THE FUNCTIONAL AND EVOLUTIONARY IMPLICATIONS OF REDUCED IMMUNE DEFENSE DUE TO MATING IN FEMALE *DROSOPHILA MELANOGASTER*

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THE FUNCTIONAL AND EVOLUTIONARY IMPLICATIONS OF REDUCED IMMUNE DEFENSE DUE TO MATING IN FEMALE *DROSOPHILA MELANOGASTER*

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Trade-offs between immune defense and life-history traits have the potential to dramatically alter the function and evolution of both traits involved. Female *Drosophila melanogaster* show reduced immune defense after mating, suggesting a potential trade-off between mating or consequent reproduction and immune defense. In this work, I demonstrated that post-mating systemic immunosuppression varies in degree across different bacterial pathogens. I also showed that the effect of mating on immune defense is genetically variable in degree among female flies, but that males are not significantly genetically variable for the degree of post-mating systemic immunosuppression they elicit in their mates. My data revealed that the variation in females is likely due to recessive deleterious alleles and did not reveal evidence for an evolutionary trade-off. I determined that reduced immune defense after mating is dependent on seminal fluid transfer and on the presence of a female germline. Consistent with this result, I identified a family of genes involved in formation of the vitelline membrane that are more reduced in expression after infection in virgin females than in mated females. These results together are consistent with a model in which virgin females reduce reproductive effort to favor immune defense, an action that may not be adaptive or physiologically possible for mated females. I identified many immune genes that are differentially altered by infection in virgin versus mated females. These included a family of antimicrobial peptide genes, genes in the *Turandot* family, and *TepII,* all of which have more strongly increased expression in virgin than

in mated females in response to infection. In a separate experiment, I found that when females are infected with bacteria, virgins have higher antimicrobial peptide transcript levels at early time points in the infection. It is possible, then, that mating alters females' capacity for systemic immune defense by directly or indirectly reducing their immune system activity.

BIOGRAPHICAL SKETCH

Sarah Short was born in lovely Willoughby, OH a quaint suburb of the infamous Cleveland, OH. She first developed an interest in science as a child through a healthy obsession with tropical rainforests and big cats. She envisioned an exciting career working in a laboratory high in the trees and studying jaguars in the jungle. Though her career path has veered in a slightly different direction, she maintains a fascination with the natural world and a fondness for tree houses. During her time in Willoughby, Sarah took an advanced placement biology course at Willoughby South high, where her interest in the biological sciences was cemented. She decided to pursue a degree in biology, but initially anticipated going to medical school after finishing her undergraduate degree.

She left Willoughby to attend college at Xavier University in Cincinnati, OH, where she majored in Biology. She found her experience at Xavier enriching and memorable. The liberal arts curriculum exposed her to many new areas of study and ways of thinking and the focus on social justice at Xavier fundamentally changed the way she viewed the world and her place in it. The biological education she received at Xavier was excellent, and she particularly remembers enjoying her classes in genetics and evolution. These two courses, along with her first research experience, made her reconsider her decision to go to medical school.

Before beginning her senior year of college, Sarah participated in a summer research program at the Cleveland Clinic working with Dr. David Hicks. Her project involved identifying diagnostic molecular markers in breast cancer and led to a first author publication. Dr. Hicks was an excellent mentor, affording Sarah a great deal of guidance but also substantial freedom to work out problems and form new ideas. Sarah was surprised to find how much she enjoyed research, and after graduating from Xavier in 2005 with a B.S. in Biology, decided to apply for graduate school to study evolutionary genetics.

Sarah came to Cornell in the fall of 2006, where she joined the laboratory of Dr. Brian Lazzaro in the Field of Genetics and Development. She became interested in immunity as a whole-organism phenotype and the implications that interactions between immune defense and life-history traits would have for the evolution and function of immune defense. She intends to extend these interests to mosquitoes in the laboratory of George Dimopoulos at the Johns Hopkins Bloomberg School of Public Health, where she has accepted a position as a post-doctoral researcher.

For Matty

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I would also like to thank my family. My parents have encouraged me every step of this long journey and have been a constant source of reassurance and support. They have always loved me for who I am and I have always known I could count on them for anything, two things I will always be grateful for. My older brother Tim never misses an opportunity to tell me how proud he is of me and my little brother Stephen has brightened my day countless times during our long chats on the phone. My sister Anna has always been there for me, and understands me in a way that only a sister can. I am incredibly grateful to have had her support the past six years.

Finally, I would like to thank my partner and best friend, Matt, who has motivated and supported me throughout my Ph.D. He has made every difficult part of the past six years more

bearable and every fun part that much more wonderful. He also makes me laugh. A lot. He has a genuine love for science and has, on a regular basis, helped me remember why I became a scientist in the first place. He inspires me every day, and I am immensely thankful to have him in my life.

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Chapter 1.

Introduction: Understanding the function and evolution of insect immune defense in the context of non-canonical immune defense factors.

Insects, like all other animals, are continuously at risk for pathogenic infection, and failure to adequately fight infection by pathogens can lead to markedly decreased fitness. For this reason, selection for an effective immune defense is predicted to be quite strong, and many *Drosophila* genes involved in immune defense show evidence of positive selection (e.g. Schlenke and Begun, 2003; Lazzaro, 2005; Schlenke and Begun, 2005; Jiggins and Kim, 2007; Sackton et al., 2007; Obbard et al., 2009; Keebaugh and Schlenke, 2012). Additionally, under laboratory conditions it is possible to artificially select for strong defense against, for example, parasitic wasps (Kraaijeveld and Godfray, 1997) and a highly virulent bacterial pathogen (Ye et al., 2009). And yet, infection persists at appreciable rates in wild *Drosophila*, and the efficacy of immune defense is highly variable within populations of wild flies (Lazzaro et al., 2004; Tinsley et al., 2006). The evolution of a highly effective immune defense can be costly, and natural selection will therefore only favor improved immune defense if it provides fitness benefits that outweigh its costs. Variation in environmental factors such as pathogen prevalence or virulence can cause the strength of selection for improved immune defense to fluctuate. The costly nature of immune defense coupled with this environmental variability can act to maintain genetic variation for immune defense.

In addition to genetic variation, immune defense can be highly variable within individuals or genotypes, and can change dramatically with alterations in physiology, life-history or the environment (Lazzaro and Little, 2009). If the immune system were cost-free, we would expect it to be constitutively active rather than induced by infection. However, deploying the immune system to fight infection can cause reduced performance of organisms for certain life-history traits, which can lead to lowered fitness (Schmid-Hempel, 2003). These antagonistic interactions can have dramatic effects on immune defense, and I therefore consider traits

involved in these interactions as "non-canonical immune defense factors." In order to fully understand how immune defense functions, it is crucial to expand our knowledge of defense to include the influence of these interactions. If they have a genetic basis and an effect on fitness, they may also act to maintain genetic variation for defense (Roff, 2002). In this dissertation, I focus on these non-canonical immune system factors and their roles in determining (1) how immune defense functions at the whole-organism level and (2) how genetic variation for immune defense is maintained. The approach I chose unites the fields of *Drosophila* immunity and ecological immunology. Researchers of *Drosophila* immunity have taken a mainly genetical approach to develop an extensive knowledge of invertebrate immune system function, and ecological immunology has sought to understand immune defense in an ecological and evolutionary context.

Trade-offs and immune defense:

Genetic or physiological interactions between immune defense and life history traits can result in trade-offs. My dissertation focuses specifically on the trade-off between immune defense and reproduction in female *Drosophila melanogaster*. Most basically, traits are said to trade off when the increased activity or performance of one results in the decreased performance of the other (Roff, 2002). Trade-offs can be evolutionary or physiological (Schmid-Hempel, 2003) and there is evidence that *D. melanogaster* immune defense is involved in both. In an evolutionary trade-off, evolving improved performance of a trait results in an evolved obligate associated cost. For example, Ye et al. (2009) showed that artificial selection for increased survival of bacterial infection in *D. melanogaster* results in a concomitant reduction in egg viability, suggesting antagonistic pleiotropy between immune defense and egg quality.

Evolutionary trade-offs necessarily have a genetic basis, occur at the population level, and can act to maintain genetic variation for immune defense and life history traits (Roff, 2002). In an evolutionary trade-off, the immune system does not need to be induced to incur a cost. An allele that confers low egg quality, for example, will do so regardless of whether an individual carrying that allele ever has to fight an infection. This is in marked contrast to physiological trade-offs.

Physiological trade-offs involving immune defense occur at the level of individuals rather than populations and arise when the use of the immune system (*e.g.* to fight an infection) incurs a cost. For example, female *D. melanogaster* that successfully melanize a parasitoid egg as larvae lay fewer eggs as adults (Fellowes et al., 1999), suggesting that the use of the immune system directly or indirectly reduces reproductive output. Additionally, mating reduces the ability of females to fight infection by pathogenic bacteria, suggesting a physiological trade-off between mating and immune defense (Fedorka et al., 2007; Short and Lazzaro, 2010). Immune defense and reproduction may trade off because they are in competition for a limited pool of resources or because they are antagonistically controlled by a shared molecular signaling pathway (Sheldon and Verhulst, 1996).

Physiological and evolutionary trade-offs are not mutually exclusive, and individual-level physiological trade-offs can underlie population-level evolutionary trade-offs. Physiological trade-offs that are independent of an evolutionary trade-off have important implications for the function of immune defense, but are not predicted to have evolutionary implications. A number of studies have demonstrated ways in which trade-offs affect the function and evolution of defense, but before discussing those studies, it is important to first define immune defense and to outline what is known about the canonical immune system.

Defining immune defense:

Throughout this dissertation, I identify immune defense as the ability to eliminate or survive infection by a pathogenic bacterium. I have chosen this end-point phenotype because it provides an assessment of the overall performance of the organism and because it is an obvious target of natural selection. Immune defense is also commonly assessed by measurement of immune system activity, such as humoral immune system signaling or phenoloxidase activity (Adamo, 2004; see below for an explanation of these terms). These so-called "proximal measures of immune defense" are valuable because they can inform how a life-history trait mechanistically interacts with immunity. However, increased activity of proximal immune traits does not always correlate with increased ability to resist or survive infection. For example, mating increases antimicrobial peptide gene expression in female *D. melanogaster,* but this does not correlate with an increased ability to fight infection (Fedorka et al., 2007; Wigby et al., 2008). Additionally, components of the immune system can trade off with other immune system processes. In these instances, measuring the performance of one aspect of immunity will not reflect the capability of the entire immune system (*e.g.* Cotter et al., 2004). For these reasons, it is not sufficient to use only proximal measures of immune system activity to gauge overall immune defense (Adamo, 2004; Lawniczak et al., 2007). In this dissertation, I measure immune defense at a whole-organism level and then assess underlying changes in proximal immune system activity. This first establishes the ecological and evolutionary relevance of the immune defense phenotype, and then provides crucial insight into the mechanistic underpinnings of whole-organism defense.

While my definition of immune defense is seemingly straightforward, there are many additional conceptual considerations that are important to keep in mind when interpreting

measurements of immune defense (Råberg et al., 2009; Viney et al., 2005). Two that are of particular importance are the concepts of tolerance and optimal immunocompetence. Immunologists have recently begun to consider defense to be a combination of resistance, *i.e.* the ability to eliminate a pathogenic threat, and tolerance, *i.e.* the ability to maintain high health and reproductive fitness in the presence of a pathogen (Råberg et al., 2009; Ayres and Schneider, 2011). This has important implications for interpreting changes in end-point phenotypes such as survival. For example, hosts may achieve improved survival either by reducing pathogen load or by increasing their tolerance of a given pathogen burden.

It is also important to consider that an optimal defense strategy is not necessarily one that is most effective at eliminating a pathogenic threat but rather one that maximizes fitness (Viney et al., 2005). This concept of "optimal immunocompetence" is especially pertinent to the examination of trade-offs between immune defense and life-history traits because it provides an adaptive framework in which to interpret the perceived costs of trade-offs. Reduced immune system activity due to a life-history trade-off may seem obviously costly, but consider that many of the compounds produced by the immune system to destroy pathogens such as reactive oxygen species also have toxic effects on host cells (Nappi and Ottaviani, 2000). For this reason and others, it may be more adaptive to have a moderate immune defense rather than a maximal immune defense. Incorporating measures of fitness into our assessments of immune defense will help indicate what level of immune defense is optimal, and remaining cognizant of optimal immunocompetence when interpreting immune defense data will improve our ecological and evolutionary understanding of immune defense (Viney et al., 2005).

The canonical immune system of *Drosophila melanogaster*:

The innate immune system consists of multiple components, each of which performs a characteristic function. The humoral immune response and the cellular immune response are considered to be the two main arms of the insect immune system. The humoral immune response primarily involves the production of antimicrobial peptides (AMPs) and the cellular immune response mainly functions by phagocytosis and encapsulation (Figure 1.1). These processes are complemented by other immune processes such as melanization and the production of cytotoxic compounds like reactive oxygen species (Figure 1.1). Each component of the immune system is most simply understood in the context of its primary function, but no arm of the immune response acts in isolation from the rest of the immune system. An in-depth discussion of the nuances of immune system regulation is beyond the scope of this introduction, but many instances of co-regulation have been documented and are worth noting (e.g. Ligoxygakis, 2002; Takehana et al., 2002; Garver et al., 2006; Tanji et al., 2007).

The humoral immune response is by far the most well-understood arm of the immune system in *Drososphila* (for in-depth reviews see Lemaitre and Hoffmann, 2007; Ferrandon et al., 2007; Wang et al., 2006). Humoral immune system signaling takes place in the fat body of the fly, and functions primarily in the production and secretion of multiple AMPs into the hemolymph (Kylsten et al., 1990; Tryselius et al., 1992; Reichhart et al., 1992; Dimarcq et al., 1994; Levashina et al., 1995; Charlet et al., 1996; Dushay et al., 2000). When bacteria or fungi are present in the hemocoel, their cell wall components are detected by proteins known as pattern recognition receptors (PRRs), which are capable of differentially recognizing Lys-type peptidoglycan (found in most gram-positive bacteria), DAP-type peptidoglycan (found in gram-negative and gram-positive bacillus species), and β -1,3-glucan (found in fungi) (Guan and

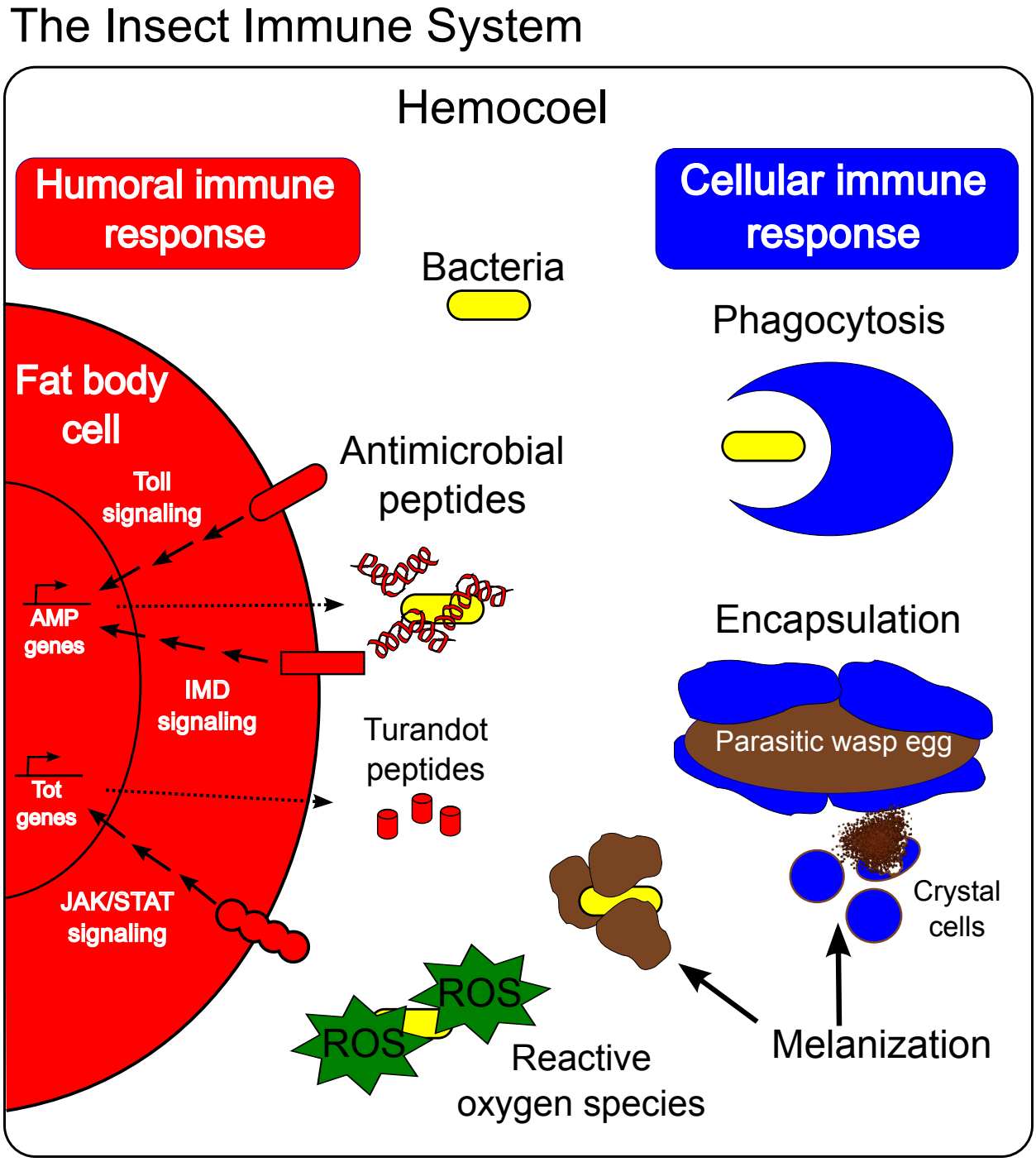

Figure 1.1: The immune system of *Drosophila melanogaster*. The two primary arms of the insect immune system are the humoral immune response and the cellular immune response. The cellular response (shown in blue) involves phagocytosis of bacteria and encapsulation of parasitoid wasp eggs by different types of hemocytes. Phagocytosis is the major function of the cellular immune response in adult flies. The humoral immune response (shown in red) primarily involves the production of antimicrobial peptides in the fat body of the fly. The presence of

bacteria in the hemolymph is detected by receptor molecules which trigger activation of the Toll or IMD pathways. Signaling via both pathways results in an increase in transcription of AMP genes. The JAK/STAT pathway also functions in the fat body and produces Turandot peptides in response to stress and wounding. A number of other components of the immune system (melanization, production of reactive oxygen and nitrogen species) do not clearly belong to the cellular or humoral immune response. For example, in larvae, the melanization response is mediated by special hemocytes called crystal cells, which are especially important for melanization of parasitoid wasp eggs. However, melanin is also made in response to bacterial infection in adults who lack crystal cells. Melanization is regulated by components of the Toll pathway, further blurring the line between the humoral and cellular immune system. These components are shown in green and brown to indicate that they are not obviously humoral or cellular.

Mariuzza, 2007; Pal and Wu, 2009). PRRs that recognize Lys-type peptidoglycan primarily activate an immune pathway that functions through the Toll receptor, as do PRRs that recognize β-1,3-glucan, while PRRs that recognize DAP-type peptidoglycan primarily activate the pathway that functions through IMD (Guan and Mariuzza, 2007). The Toll and IMD pathways are the major signaling pathways in the humoral immune response, and their activation results in dramatic increases in transcription of antimicrobial peptide genes (Ferrandon et al., 2007). Notably, not all PRRs increase humoral immune system signaling. Three of the 13 known peptidoglycan receptors (a specific type of PRR) act to regulate the humoral immune system by decreasing signaling (Bischoff et al., 2006; Zaidman-Rémy et al., 2006). A third, less well-studied pathway in the humoral immune system is the JAK/STAT pathway, which is not implicated in the production of antimicrobial peptides, but rather in induction of the expression of genes in the *Turandot* (*Tot*) family (Agaisse and Perrimon, 2004). The function of *Tot* genes is not yet known, but they are induced by septic injury and may be important in general stress response (Ekengren and Hultmark, 2001).

The cellular immune response is so named because it is carried out by insect blood cells, called hemocytes (Williams, 2007). It involves the phagocytosis of bacteria by plasmatocytes (a specific kind of hemocyte) and, in *Drosophila* larvae, also involves the encapsulation and melanization of parasitoid wasp eggs by lamellocytes and crystal cells, respectively (Williams, 2007). Phagocytosis is the primary activity of the cellular immune response in adult flies, and multiple receptors have been found to be important for this function in plasmatocytes. These include *scavenger receptor class C type 1* (Rämet et al., 2001), *PGRP-LC* (Rämet et al., 2002), *eater* (Kocks et al., 2005) and the *nimrod* gene family (Kurucz et al., 2007). Additionally, genes

in the *Tep* family have been purported to act as opsonins, which target bacterial cells for phagocytosis (Levashina et al., 2001).

In addition to the production of antimicrobial peptides, insects also engage in the production of a number of compounds that are damaging to pathogenic cells. For example, upon infection or wounding, the fly produces melanin, which involves the phenoloxidase-regulated conversion of phenols to quinones, which then oligomerize to form melanin (Cerenius and Söderhäll, 2004). Melanin has antimicroabial properties, as do the intermediates formed during its production (Cerenius and Soderhall, 2004). In addition to melanin, reactive oxygen and reactive nitrogen species are produced during an infection in the fly (Nappi and Ottaviani, 2000), especially in epithelial tissues, such as the gut (Ha et al., 2005).

The extensive functional understanding of the canonical immune system in insects, particularly in *D. melanogaster,* has provided crucial knowledge to our understanding of overall immune defense. Despite this knowledge, we are still lacking in ability to account for the widespread genetic variation and physiological variability in immune defense. One potential explanation for the variation in immune defense lies within interactions between the canonical immune system and costly life-history traits, which can act to alter the function of immune defense and can act to maintain genetic variation for defense.

Physiological trade-offs and insect immune defense:

Since Sheldon and Verhulst's (1996) seminal paper suggesting that immune defense should be viewed in the context of costly life-history traits, many studies have provided evidence for physiological trade-offs between immune defense and fitness-related traits in insects. Physiological trade-offs are inferred to be present when increased activity of one trait correlates

with the reduced performance of another trait. They have the potential to alter the function of the immune system and an organism's overall ability to fight infection (Schmid-Hempel, 2003). In order to test for a physiological trade-off, most commonly a treatment is applied (*e.g.* infection) that experimentally increases the investment of an organism in one trait (immune defense), and the effect of that treatment on other traits (*e.g.* egg number) is observed. Using this general approach, multiple life-history and ecological traits have been shown to trade off with immune defense including mating and consequent reproduction, longevity and development.

Physiological trade-offs between immune defense and reproduction: In *Aedes aegypti* mosquitoes, infection by *Plasmodium gallinaceum* results in decreased female fecundity (Hacker and Kilama, 1974). Melanization of beads injected into the *Ae. aegypti* hemocoel also results in decreased reproductive output, although the extent varies depending on the charge of the injected beads (Schwartz and Koella, 2004). Infection with *Plasmodium falciparum* causes reduced fecundity in female *Anopheles gambiae* mosquitoes (Hogg and Hurd, 1997). Challenge of *Anopheles gambiae* with lipopolysaccharide, a generic immune elicitor, results in increased apoptosis of follicle cells surrounding oocytes (Ahmed and Hurd, 2006). Both findings suggest a reproductive cost of utilizing the immune system. In this same species, Rono et al. (2010) used RNAi to knockdown vitellogenin and lipophorin protein levels in females, which are both crucial for oogenesis. This dramatically hindered oogenesis and simultaneously increased the efficacy of the immune response against *Plasmodium berghei*, the parasite responsible for rodent malaria (Rono et al., 2010). This work suggests a potential mechanism for trade-offs between reproduction and defense against *Plasmodium* infection in mosquitoes.

In the dung beetle *Euoniticellus intermedius*, activation of the immune system by a generic immune elicitor results in reduced female reproductive output (Reaney and Knell, 2010),

and in the dampwood termite *Zootermopsis angusticollis,* experimentally inducing a melanization response in females results in delayed onset of oviposition (Calleri et al., 2007). Additionally, in the leaf-cutting ant *Atta colombica*, increased sperm storage results in decreased capacity for encapsulation and melanization (Baer et al., 2006), and in damselflies, increases in oviposition correlates with decreased encapsulation ability (Siva-Jothy et al., 1998). In *Drosophila melanogaster,* adult females that have successfully defended themselves from parasitoid wasp attack produce fewer eggs compared to unchallenged adult females (Fellowes et al., 1999).

Many studies show a mating-induced reduction in phenoloxidase activity, which is important for melanization. For example, mated dampwood termite females have reduced melanization capacity compared to virgin controls, but only when they are engaged in the production of mature oocytes (Calleri et al., 2007). In the cricket *Acheta domesticus*, mated females have a reduced ability to melanize relative to virgin controls (Bascuñán-García et al., 2010), and in the mealworm beetle *Tenebrio molitor*, mating results in a decrease in phenoloxidase activity in both male and female beetles (Rolff and Siva-Jothy, 2002). Rolff and Siva-Jothy hypothesized that juvenile hormone, which is known to increase after mating in insects, may mediate this effect. They were able to recapitulate the effect of mating on immunity by transplanting *corpora allata* (the organs responsible for synthesizing juvenile hormone) from mated individuals into unmated individuals, and were able to eliminate the effect of mating by introducing a juvenile hormone inhibitor. These combined results led them to conclude that increased juvenile hormone titer after mating likely decreased phenoloxidase activity. This effect is not generalizable across all sampled invertebrates, however, as female *Allonemobius socius* crickets show reduced hemocyte numbers, lytic activity and encapsulation ability, but increased

phenoloxidase activity after mating, suggesting a mating-induced reduction in constitutive immune system activity that may exclude phenoloxidase activity in this organism (Fedorka et al., 2004).

In *D. melanogaster,* after high levels of sexual activity males have a reduced ability to clear non-pathogenic bacteria injected into the hemocoel relative to males not engaged in sexual behavior (Mckean and Nunney, 2001). In female *D. melanogaster*, mating results in a reduced ability to eliminate and survive infection by multiple bacterial pathogens (Fedorka et al., 2007; Short and Lazzaro, 2010) but does not significantly alter females' abilities to clear non-pathogenic bacteria injected into the hemocoel (McKean and Nunney, 2005; Wigby et al., 2008).

Physiological trade-offs between immune defense and longevity: In the bumblebee *Bombus terrestris*, activation of phagocytosis or the humoral immune response results in reduced overall survival, but bees are able to eliminate this trade-off by consuming more food (Moret and Schmid-Hempel, 2000). Female *Tenebrio molitor* beetles and female *Acheta domesticus* crickets have reduced longevity after melanizing a nylon filament inserted into the hemocoel (Armitage et al., 2003; Bascuñán-García et al., 2010). *D. melanogaster* females that have constitutively upregulated signaling of the humoral immune system (DeVeale et al., 2004; Libert et al., 2006) also show reduced longevity. These studies together suggest a longevity cost of utilizing the immune response.

Physiological trade-offs between immune defense and growth/stress response: In *D. melanogaster,* males and females that have successfully defended themselves from parasitoid wasp attack as larvae are smaller as adults (Fellowes et al., 1999). *Acheta domesticus* crickets also suffer reduced body size with increased melanization response (Bascuñán-García et al., 2010). Additionally, starvation and dessication resistance is reduced in *D. melanogaster* that

were parasitized as larvae compared to adults that were not parasitized (Hoang, 2001). In the wax moth *Galleria mellonella*, microbial challenge before and during pupation results in a significant decrease in development time in both male and female moths (Meylaers et al., 2007). These examples suggest the possibility of a trade-off with immune defense, but it is also possible that the reductions in growth and stress response observed in these studies are instead a result of the pathology of the infection.

Instances of demonstrated absence of a physiological trade-off:

Life history traits do not always trade off against defense. In fact, researchers in multiple studies have failed to detect trade-offs, suggesting that they may not be as ubiquitous as is often assumed. In the yellow dung fly *Scathophagia stercoraria*, for example, mating does not alter phenoloxidase activity in males or females (Schwarzenbach et al., 2005), and in the cricket *Gryllus texensis*, females that have mated are better able to survive infection by the bacteria *Serratia marcescens* compared to virgin controls (Shoemaker et al., 2006). These results seem to indicate that immune defense incurs no cost in these species. It has been suggested that the likelihood of a trade-off may depend on the nature of the interaction between the two traits, especially the physiological mechanism by which immune defense is altered (Rigby et al., 2002). However, environmental factors have the potential to mask detection of immune defense trade-offs (Sandland and Minchella, 2003). Laboratory conditions are generally much less challenging than what an organism would face in the wild. It is possible that exposing insects from these species to more harsh environmental conditions, by manipulating food availability for example, would reveal immune trade-offs.

Evolutionary trade-offs and insect immune defense

Far fewer studies have demonstrated the presence of evolutionary trade-offs involving immune defense, perhaps because it is more difficult to test for them than it is to test for physiological trade-offs (Schmid-Hempel, 2003). There are two common ways to test for an evolutionary trade-off. The first is to artificially select for improved immune performance and then to test for reduced performance of life-history traits in the selected lines under non-immune challenge conditions. A correlated response to selection suggests a cost of evolving improved immune defense. The second is to measure standing genetic variation for each trait hypothesized to be involved in an evolutionary trade-off and then to test for a negative genetic correlation between traits, as this would be predicted in the case of antagonistic pleiotropy. Studies testing for evolutionary trade-offs between immune defense and life-history traits have successfully utilized both of these methods to identify a number of putative evolutionary trade-offs.

In the yellow dung fly *Scathophaga stercoraria,* selection for increased phenoloxidase levels results in decreased longevity in starvation conditions, suggesting that in stressful circumstances, there is a survival cost of having high circulating phenoloxidase (Schwarzenbach and Ward, 2006). Additionally, *Aedes aegypti* selected for earlier pupation time show reduced melanization capacity, while those selected for later pupation time have higher melanization abilities (Koella and Boete, 2002). This negative genetic correlation between development time and melanization suggests an evolutionary trade-off between the two traits.

In *Drosophila melanogaster*, female genotypes with high resistance to bacterial infection have lower early life fecundity in the absence of infection, suggesting an evolutionary trade-off between resistance and reproduction (McKean et al., 2008). Additionally, flies selected for increased survival of attack by parasitoid wasps are less able to forage in food-limited

environments compared to non-selected controls (Kraaijeveld and Godfray, 1997; Fellowes et al., 1998). It was later determined that lines selected for increased resistance to parasitism have approximately twice as many hemocytes as unselected controls, which may explain why the selected lines are better able to survive parasitoid attack (Kraaijeveld et al., 2001). Lastly, selection for improved ability to survive infection by the highly virulent bacteria *Pseudomonas aeruginosa* results in a concomitant reduction in development time and egg viability as well as a female-specific reduction in longevity (Ye et al., 2009).

Mating and immunity in female *Drosophila melanogaster*

The specific trade-off that I focus on in the following dissertation chapters is that between immune defense and mating/reproduction. To summarize, mating reduces the ability of female *D. melanogaster* to fight infection by pathogenic bacteria (Fedorka et al., 2007; Short and Lazzaro, 2010; Short et al., 2012, this dissertation) and females that successfully melanize a parasitoid egg as larvae lay fewer eggs as adults (Fellowes et al., 1999). These physiological trade-offs, coupled with the potential evolutionary trade-offs between reproduction and defense that have been identified in female *Drosophila* (McKean et al., 2008; Ye et al., 2009) suggest that reproduction may interact with immune defense on a physiological level to affect the function and efficacy of defense and on a genetic level to affect the evolution of defense. Beyond these studies, however, little is known about the nature of the putative physiological or genetic interactions between mating and immune defense.

A number of studies have identified mating-responsive immune system genes, such as the antimicrobial peptide genes, which suggests a connection between mating and humoral immune defense. These studies identified increased transcript abundance of AMP genes (McGraw et al.,

2004; Lawniczak and Begun, 2004; Peng et al., 2005; Fedorka et al., 2007; Wigby et al., 2008; Innocenti and Morrow, 2009), which seems paradoxical given the decrease in overall systemic immune defense that occurs after mating (Fedorka et al., 2007; Short and Lazzaro, 2010). Gene expression studies of reproductive tissue demonstrate that mating-induced increases in transcript abundance for antimicrobial peptide genes occurs in the lower female reproductive tract (Mack et al., 2006; Kapelnikov et al., 2008; Wigby et al., 2008; Fedorka et al., 2007). Whether it also occurs in other tissues remains an open question. Peng et al. (2005) showed that induction of AMP genes is at least in part driven by transfer of the male accessory gland protein sex peptide, and that sex peptide acts to control AMP expression via the systemic humoral immune response pathways. AMP expression in the female reproductive tract is not dependent on the systemic humoral immune response pathways (Ferrandon et al., 1998; Tzou et al., 2000), so this suggests that mating and sex peptide may alter AMP expression in tissues outside the reproductive tract. Domanitskaya et al. (2007) reported that SP transferred during mating alters *drosocin* gene transcription in the oviduct but did not report testing for other tissue-specific expression. It is therefore unclear whether post-mating increases in AMP gene expression are wide-spread or tissue-specific. Regardless, these mating-responsive changes in AMP gene expression do not explain mating-induced reductions in overall defense, and the question of how mating acts to reduce defense against systemic infection remains unanswered.

In this dissertation, I investigate the functional and evolutionary implications of the suppressive effect that mating has on immune defense. In Chapter 2, I determine that mating results in reduced overall immune defense and that this post-mating immunosuppression is genetically variable. In Chapter 3, I test whether improved immune defense correlates with reduced egg or progeny production, which might explain the genetic variation observed in

Chapter 2. Rather than an evolutionary trade-off, I find that genetic variation in defense and fecundity likely arises from recessive deleterious mutations that affect overall resource allocation and/or general vigor. In Chapter 4, I identify a role for seminal fluid transfer and egg production on immune defense and also find that antimicrobial peptides are less induced in infected mated females relative to infected virgins. In Chapter 5, I investigate genome-wide changes in transcript abundance due to mating and infection. I find a number of genes that are differentially affected by infection in virgins compared to mated females, including a large family of genes involved in egg production. This dissertation reveals a vital role of mating status and reproduction on the efficacy of defense and elucidates the ways in which immunity and reproduction are physiologically intertwined. Additionally, it demonstrates the unequivocal importance of incorporating non-canonical immune defense factors into our understanding of the function and evolution of immune defense.

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Chapter 2.

Female and male genetic contributions to post-mating immune defense in female *Drosophila melanogaster*.

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Abstract:

Post-mating reduction in immune defense is common in female insects, and a trade-off between mating and immunity could affect the evolution of immunity. In this work, we tested the capacity of virgin and mated female *Drosophila melanogaster* to defend against infection by four bacterial pathogens. We found that female *D. melanogaster* suffer post-mating immunosuppression in a pathogen-dependent manner. The effect of mating was seen after infection with two bacterial pathogens (*Providencia rettgeri* and *Providencia alcalifaciens*), though not after infection with two other bacteria (*Enterococcus faecalis* and *Pseudomonas aeruginosa*). We then asked whether the evolution of post-mating immunosuppression is primarily a 'female' or 'male' trait by assaying for genetic variation among females for the degree of post-mating immune suppression they experience and among males for the level of post-mating immunosuppression they elicit in their mates. We also assayed for an interaction between male and female genotypes to test the specific hypothesis that the evolution of a trade-off between mating and immune defense in females might be being driven by sexual conflict. We found that females, but not males, harbor significant genetic variation for post-mating immunosuppression, and we did not detect an interaction between female and male genotypes. We thus conclude that post-mating immune depression is predominantly a 'female' trait, and find no evidence that it is evolving under sexual conflict.

Introduction:

Immune defense (defined as the combined ability of an organism to both actively fight and to tolerate an infection (Ayres & Schneider 2008) is generally considered to be costly in that its maintenance and deployment often results in physiological and evolutionary trade-offs against

other traits important for fitness, including longevity (Moret & Schmid-Hempel 2000; DeVeale *et al*. 2004; Ye *et al*. 2009), larval ability to compete for food (Kraaijeveld & Godfray 1997), body size (Fellowes *et al* 1999), fertility (Ye *et al*. 2009) and fecundity (Fellowes *et al*. 1999; McKean *et al*. 2008). It has been proposed that mating may have immunosuppressive effects in females in order to allow limited resources to be shunted from immunological requirements to reproductive needs (Sheldon & Verhulst 1996). Examples of post-mating immune depression include a reduction in phenoloxidase activity after mating in the beetle *Tenebrio molitor* (Rolff & Siva-Jothy 2002), and decreased encapsulation ability correlated with increased oviposition in damselflies (Siva-Jothy *et al*. 1998). Additionally, mating causes decreased survival after infection with a pathogen in female *D. melanogaster* (Fedorka *et al*. 2007) but see (McKean & Nunney 2005; Wigby *et al*. 2008), and increased mating effort leads to decreased hemocyte number, lytic activity and encapsulation ability in the cricket *Allonemobius socius* (Fedorka *et al*. 2004). Post-mating immunosuppression may not be universal, as it was not detected in yellow dung flies (Schwarzenbach *et al*. 2005), and phenoloxidase activity and parasite resistance are even increased after mating in female *Allonemobius socius* and *Gryllus texensis*, respectively (Fedorka *et al*. 2004; Shoemaker *et al*. 2006). Determining how and why immune defense is altered by mating (or other fitness-related activities) is crucial to our understanding of how immune defense evolves, as well as how it functions at the whole-organism level.

The immune system of *D. melanogaster* is well understood, and extensive genetic analysis has revealed many of the genes involved in the function of the humoral and cellular immune response (reviewed in Lemaitre & Hoffmann 2007). This work has mainly focused on the function of the canonical immune system; but pleiotropic connections to mating (or other costly processes) have the potential to dramatically alter or limit the function and evolution of

overall levels of defense (reviewed in Lawniczak *et al*. 2007). In the present study, we first determined whether mating affects the function of female immune defense. We utilized multiple pathogens in order to establish the generality of the phenomenon and to elucidate the potential importance of pathogen diversity on changes in defense due to mating. We were also interested in determining the role that this trade-off could play in shaping the evolution of immune defense. We therefore assessed the level of genetic variation among females for the reduction in defense they experience after mating and among males for the level of post-mating immunosuppression they elicit in their mates. We also determined whether the change in defense is dependent on the particular combination of male and female genotypes engaging in copulation. We measured genetic variation in both sexes because we were particularly interested in assessing the potential for ongoing sexual conflict in this system, as it has been suggested in the literature that the fitness of males and females may be affected differently depending on the level of immunosuppression females experience after mating, and that this could lead to sexual conflict (Fedorka *et al*. 2007; Lawniczak *et al*. 2007). This hypothesis could be provisionally supported by data we have collected, which suggest that the male ejaculate plays a role in reducing female defense (S. M. Short, M. F. Wolfner and B. P. Lazzaro 2010, unpublished data). Furthermore, components of the seminal fluid have been demonstrated to be involved in dynamic evolutionary interactions such as sexual conflict (Rice 1996)**,** and multiple proteins in the male ejaculate of *D. melanogaster* have been shown to be rapidly evolving (Swanson *et al*. 2001; Mueller *et al*. 2005). If extant genetic variation in female immunosuppression was maintained by an ongoing intersexual interaction (*e.g*., sexual conflict) in our sampled population, we could expect to observe that the magnitude of post-mating immunosuppression is determined by the specific male and female genotypes participating in a mating (Gillespie & Turelli 1989)**.** *D. melanogaster*

possess significant genetic variation for immune resistance (Lazzaro *et al*. 2004; Tinsley *et al*. 2006**)**, and variation in the immunological cost incurred by mating is one potential source of that genetic variation.

Materials and methods:

(a) Fly stocks and maintenance: We used the following lines of *Drosophila melanogaster*: Canton S (a wild-type inbred strain), and 18 lines chosen randomly from the Drosophila Genetic Reference Panel (DGRP), a collection of inbred isofemale lines collected in Raleigh, NC (Ayroles *et al*. 2009). Each line is genetically distinct and the total set represents a "snapshot" of naturally occurring genetic variation in this population at the time of sample. Nine DGRP lines (coded 1F-9F) were used to assay female variation. These were RAL-324, RAL-362, RAL-820, RAL-639, RAL-375, RAL-315, RAL-437, RAL-786 and RAL-486. Nine different DGRP lines (coded 1M-9M) were used to assay male variation. These were RAL-391, RAL-774, RAL-358, RAL-303, RAL-380, RAL-712, RAL-732, RAL-208 and RAL-360. Flies for all experiments were reared at 24ºC on a 12 hour light-dark cycle on standard glucose medium (12g agar, 100g glucose and 100g Brewer's yeast per 1.2L of water, plus 0.04% phosphoric acid and 0.4% propionic acid (final concentration) added to inhibit microbial growth in the food).

(b) Experimental design: To test the effect of mating on bacterial load and survival after infection with multiple pathogens, we conducted multiple experiments (one per pathogen) each in a complete block design, where both virgin and mated females were assayed for either bacterial load or survival in each replicate of the experiment. To test for genetic variation across lines from the DGRP, we used females from nine lines (coded 1F-9F) and males from nine additional lines (coded 1M-9M). The experiment was conducted in a manner similar to a lattice

square design, with minor departures from classical set-up due to experimental contingencies. Bacterial load data for virgin and mated females was collected for all 81 pairwise crosses between all nine "F" lines and all nine "M" lines, with the entire experiment conducted in duplicate. Due to the labor involved in assaying infection phenotypes in a crossing scheme of this scale, we opted to measure only the bacterial load phenotype in this part of the experiment. We feel that this is justified in that mated females sustain significantly higher bacterial loads and significantly higher mortality than virgins do after infection with *P. rettgeri* (Figures 2.1a, 2.2a), so either phenotype is a reliable indicator of overall defense. Additionally, because of the magnitude of the experiment, data for all of the 81 pairwise crosses (comprising a single replicate of the entire experiment) was collected over 9 days. On each day, nine of the 81 pairwise combinations were observed, with females from each "F" line mated to males from a single, randomly assigned "M" line, such that all "F" lines and all "M" lines were used each day. At the end of the nine-day experiment, all "F" lines had been paired to all "M" lines once, with data for virgin and mated females from each of these 81 combinations recorded. The randomization scheme for this experiment was generated using the Plan procedure in SAS (SAS Institute, Cary, NC). On any given day, we shuffled the order in which each "F" line and each "M" line was mated and infected using the "sample" function in R (R Foundation for Statistical Computing, Vienna, Austria).

(c) Mating set up: All matings were set up individually between a single virgin female and a single virgin male. All flies (males and females) were collected as virgins and aged three days post-eclosion with *ad libitum* access to food in groups of ~30. The day before matings were to be set up, virgin females were anaesthetized with CO_2 and placed in individual vials containing abundant media. They were then randomly allocated to "virgin" or "mated" treatment. Females

were allowed to recover overnight. The next morning (within three hours of incubator "dawn"), unanaesthetized virgin males were aspirated into each vial assigned to the "mated" treatment and each mating was individually observed. Matings lasting less than 15 minutes were not used for the experiments testing multiple pathogens, but this lower bound was reduced to a minimum of 10 minutes in the experiment to assess genetic variation. This was done because many mating pairs in this experiment copulated for shorter times than Canton S flies, possibly due to natural variation in mating times. Lowering this boundary enabled inclusion of ~25% of our final dataset, and therefore significantly increased our sample size. The number of 10 minute matings were not equally distributed across genotypes (Chi-square test for the null hypothesis of equal distribution across lines for males: $X^2 = 228.73$, df = 8, $p < 2.2 \times 10^{16}$ and females: $X^2 = 68.5$, df = 8, $p < 9.7 \times 10^{12}$), but the average length of mating did not correlate with change in bacterial load (for all 81 genotype combinations: $r = -0.024$, $p = 0.83$), so we are confident that the inclusion of these shorter matings did not bias the results of our study. After mating, mated females were removed from the presence of males.

(d) Infection procedure: Two to three hours after mating cessation, mated females and their virgin counterparts were alternately anaesthetized in groups of 15 or fewer on CO_2 and pricked in the thorax with a needle dipped in dilute bacterial culture (see below). Females were then placed in a vial containing media to recover. A subset of flies from each mating treatment was pricked with a sterile needle as a wounding control. Bacterial species used for infection were as follows: *Providencia rettgeri* (isolated from wild-caught *D. melanogaster* by B. Lazzaro in State College, PA, USA), *Providencia alcalifaciens* (isolated from wild-caught *D. melanogaster* by P. Juneja and S. Short in Ithaca, NY, USA), *Enterococcus faecalis* (isolated from wild-caught *D. melanogaster* by B. Lazzaro) and *Pseudomonas aeruginosa* (species type strain, PAO1) (Table

2.1). *P. rettgeri, P. alcalifaciens* and *Ps. aeruginosa* are all Gram-negative bacteria, while *E. faecalis* is Gram-positive. All of these species are opportunistic pathogens with broad host ranges, and all have the ability to infect humans (Manos & Belas 2006; Yahr & Parsek 2006; Devriese *et al.* 2006). All bacterial cultures were grown overnight in Luria broth (LB) at 37ºC from a single bacterial colony. Each overnight culture was then diluted with sterile LB to $O.D._{600}$ = 1.0, with the exception of *E. faecalis*, which was diluted to $O.D._{600}$ = 0.5. This resulted in delivery of ~3×10^3 bacterial cells to each infected female with *P. rettgeri* and *P. alcalifaciens*, ~1×10^4 with *Ps. aeruginosa,* and approximately 5×10^2 with *E. faecalis*.

(e) Bacterial load assay: To assay bacterial load, females were aged after infection with *ad libitum* access to food for either 24 ± 0.5 hours (for the experiments represented in Figures 2.1 and 2.2) or 26-28 hours (for the genetic variation experiment). At this time, females were anaesthetized on CO_2 and homogenized in 500μL LB in pools of three (Figures 2.1 and 2.2) or five (genetic variation experiment). The homogenate was diluted 1:100 for *E. faecalis*, 1:1000 for *P. rettgeri*, and 1:10,000 for *P. alcalifaciens* and *Ps. aeruginosa* prior to plating. Fifty microliters of each diluted homogenate was deposited in a spiral pattern on LB agar using a WASP II spiral plater (Microbiology International, Bethesda, MD), and plates were incubated overnight at 37ºC. We verified that the colonies on the plate were of the species used for infection by visual inspection and periodic analysis of 16S rDNA. 16S rDNA was amplified from randomly selected colonies throughout the experiment using the primers fD1 and rP2, which amplify the rDNA of most eubacteria (Weisburg *et al*. 1991). PCR product from these colonies as well as from positive control colonies taken from a pure freezer stock of each bacterial species was digested with StuI and/or MspI, and the digested products were run on a 1% agarose gel. Digest patterns of colonies taken from infected females always matched those of

the pure freezer stock. Bacterial colonies were counted using the ProtoCOL plate counting system (Microbiology International, Bethesda, MD) associated with the spiral plater, allowing estimation of the number of viable bacteria present in each pool of homogenized females. These were the primary data used for analysis. Seven to sixteen data points were collected per replicate per treatment for each experiment in Figure 2.1. For the genetic variation experiment, 2-5 data points were collected per treatment per replicate for each pairwise combination, yielding a total of 4-10 data points per treatment for each pairwise combination, with the exception of 6F x 2M, for which only a single replicate was obtained. Six plates with zero colonies were excluded from analysis for the genetic variation experiment, as these zero counts could represent either an absence of bacteria in the flies or a technical error in the plating process. Since we cannot definitively say the flies contained zero bacterial cells, we chose to exclude these data points. The excluded data represent less than 0.5% of the dataset and eliminating these six data points has a negligible effect on the outcome of the analysis.

(f) Survival assay: To assay survival, females were placed in groups of ~10 after infection and observed either daily (for slower-acting pathogens like *P. rettgeri*), or at shorter intervals for the first 48 hours after infection (for fast-acting pathogens like *Ps. aeruginosa*). Females from both virgin and mated treatments were put onto fresh food every other day. Survival was observed for seven days after infection with *P. rettgeri* due to its gradually induced mortality, but only for five days for *E. faecalis* since most mortality occurs in the first 48 hours after infection with this bacterium. Survival for *P. alcalifaciens* and *Ps. aeruginosa* was observed for 48 hours or until all flies were dead.

(g) Statistical analysis: To assess the effect of mating on bacterial load, we first performed a natural log transformation on bacterial load values for each bacterial pathogen to produce data that more closely fit a normal distribution. We then performed an ANOVA for each bacterial pathogen to determine the effect of mating status on bacterial load. Assumptions for the ANOVA were evaluated by running diagnostic plots (fitted values versus residuals, residual normal quantile-quantile plot) and visually assessing heteroskadicity and normality of residuals. In cases where residuals were found to be non-normal (verified by Shapiro-Wilk test), deviation from normality was due to a few outlier points. Removal of outlier points did not change the significance of mating status, and ANOVA results were therefore considered to be robust. These analyses were performed in SAS (SAS Institute, Cary, NC).

To analyze our survival data, we used Cox regression analysis in SAS (SAS Institute) to determine the effect of mating status on survival over time. Event data were recorded for each fly (where an "event" = death), and flies not dead by the last time point recorded were treated as censored data. Survival curves were generated using the Kaplan Meier method in R (R Foundation for Statistical Computing).

To determine the level of genetic variation between lines from the DGRP, we first performed a natural log transformation on the bacterial load data collected from females from each pairwise mating combination. We then performed an analysis of variance with Proc Mixed in SAS (SAS Institute) using the following mixed model:

$$\begin{aligned} Y_{ijkl} = {} & \mu + \text{Mating status}_i + \text{Female genotype}_j + \text{Male genotype}_k \\ & + \text{Replicate Experimental Day}_l + \text{Mating status}_i * \text{Female genotype}_j \\ & + \text{Mating status}_i * \text{Male genotype}_k + \text{Female genotype}_j * \text{Male genotype}_k \\ & + \text{Mating status}_i * \text{Female genotype}_j * \text{Male genotype}_k + \varepsilon_{ijkl} \end{aligned}$$

where Y = ln(bacterial load) data taken from all females, Mating $status_i$ (i=1,2) represents whether females were virgin or mated, Female $genotype_j$ (j=1,9) represents the DGRP lines contributing females to crosses, Male $genotype_k$ (k=1,9) represents the DGRP lines contributing males to crosses and Replicate Experimental Day_l (l=1,20) is a factor including all days over which the experiment was conducted. Each replicate required 9 days, and two replicates were performed for a total of 18 days. Missing data were subsequently filled in over 2 additional days, resulting in *df* = 19 for Day in the model. The factor mating $status_i$*female $genotype_j$ tests for genetic variation among females for post-mating immunosuppression, while mating $status_i$*male $genotype_k$ tests for male genetic variation for the level of immunosuppression they elicit in their mates. Mating $status_i$*female $genotype_j$*male $genotype_k$ tests whether the effect of a particular male or female genotype on post-mating immunosuppression depends on the genotype of their mate.

Mean bacterial loads for each female and male genotype were obtained by finding the arithmetic mean of the log-transformed bacterial load data and back transforming it to obtain the geometric mean. We then calculated 95% confidence intervals (Sokal & Rohlf 1995).

Results and Discussion:

(a) Mating reduces female immune defense against two natural bacterial pathogens

It is yet unclear how ubiquitous post-mating reduction in immune defense is in insects. Many experiments testing potential trade-offs between immunity and defense have relied on indirect measures of immune quality (*e.g.* encapsulation ability, phenoloxidase activity or antimicrobial peptide gene expression) in the absence of actual infection (Siva-Jothy *et al*. 1998; Rolff & Siva-Jothy 2002; Fedorka *et al*. 2004; Lawniczak & Begun 2004; McGraw *et al*. 2004;

Peng *et al.* 2005). While informative with regard to the potential mechanisms linking mating and the immune system, these assays do not directly measure changes in the ability of an organism to resist or tolerate an infection, and must be interpreted with care (Adamo 2004). Other studies have measured overall defense as a function of mating in the context of experimental infection (McKean & Nunney 2001, 2005; Shoemaker *et al*. 2006; Fedorka *et al*. 2007; Wigby *et al*. 2008). Three of the cited studies have been performed using female *D. melanogaster* (McKean & Nunney 2005; Fedorka *et al*. 2007; Wigby *et al*. 2008), but no clear consensus has emerged even from those as to whether females suffer a meaningful reduction in immune defense after mating. Two of these studies (McKean & Nunney 2005; Wigby *et al*. 2008) show no change due to mating in the ability of females to clear nonpathogenic bacteria, while Fedorka *et al*. 2007 demonstrated that females infected with a pathogenic bacterium suffer higher mortality if they have mated. We hypothesized that the lack of consensus in this body of literature could be due to the effect of mating being dependent on the assay used to measure defense and/or the microbe used to test changes in defense (for example, pathogenic versus non-pathogenic infection). We, therefore, tested the effect of mating on female immune defense using two different assays (survival and systemic bacterial load) and four pathogens that differ in biology and pathogenicity (Table 2.1).

We infected female *D. melanogaster* of the strain Canton S with each of the four bacterial pathogens in Table 2.1, 2-3h after mating cessation. We also infected virgin females in parallel to serve as a control comparison. We then assayed bacterial load (*i.e*. the number of colony forming units present in a fly) and survival in mated and virgin females after infection with each bacterial species. Females pierced with a sterile needle yielded zero bacterial colonies and had negligible

Table 2.1. Pathogens that vary in biology and virulence were used for infection of virgin and mated female *D. melanogaster.* Percent mortality is averaged across virgin and mated females, and natural pathogens are those that have been isolated from the hemolymph and/or thoracic muscle of wild-caught *D. melanogaster* (see Materials and methods for details).

pathogen	virulence level	natural pathogen
P. rettgeri	moderate (~ 40% mortality)	yes
P. alcalifaciens	high (~ 98% mortality)	yes
E. faecalis	moderate (~ 60% mortality)	yes
Ps. aeruginosa	high (100% mortality)	no (strain PAO1)

mortality. At 24 hours after infection with *Providencia rettgeri* (Figure 2.1a) or *Providencia alcalifaciens* (Figure 2.1b), we observed significantly higher bacterial loads in mated females compared with virgin females (*P. rettgeri*: $p < 0.0001$, *P. alcalifaciens*: $p = 0.0024$). We also observed significantly reduced survival in mated females compared to their virgin counterparts after infection with either *P. rettgeri* (Figure 2.2a, $p < 0.0001$) or *P. alcalifaciens* (Figure 2.2b, $p < 0.0001$). In contrast, we observed no difference in bacterial load due to mating after infection with either *Enterococcus faecalis* (Figure 2.1c, $p = 0.279$) or *Pseudomonas aeruginosa* (Figure 2.1d, $p = 0.6804$), and no effect of mating on survival after infection with *E. faecalis* (Figure 2.2c, $p = 0.0811$) or *Ps. aeruginosa* (Figure 2.2d, $p = 0.3466$). Virgins infected with *E. faecalis* had a slightly (but not significantly) higher probability of survival at multiple time points (*e.g.* mean percent survival at five days post infection for virgin = 46.5% and for mated = 37.6%), but this effect was apparent in only two of the four replicates in the experiment (difference between treatments in two of the four replicates considered alone: $p = 0.0164$; in the other two replicates alone: $p = 0.8587$).

Our data show that mating results in reduced defense for females after infection with at least two pathogenic species of bacteria, both of which are pathogens of wild *D. melanogaster*. These results, coupled with previous findings showing no effect of mating in females after infection with a non-pathogenic bacterium (McKean & Nunney 2005; Wigby *et al*. 2008), suggest that, while general immune maintenance and immunocompetence are not impaired after mating, the ability of females to defend against pathogenic infection is hindered. Interestingly, and in contrast to our study, Fedorka *et al*. 2007 also used *Ps. aeruginosa* and *did* detect post-mating immunosuppression, suggesting that the magnitude of the effect may vary over bacterial strains, host genotypes, or experimental conditions. Nevertheless, the total data suggest that the

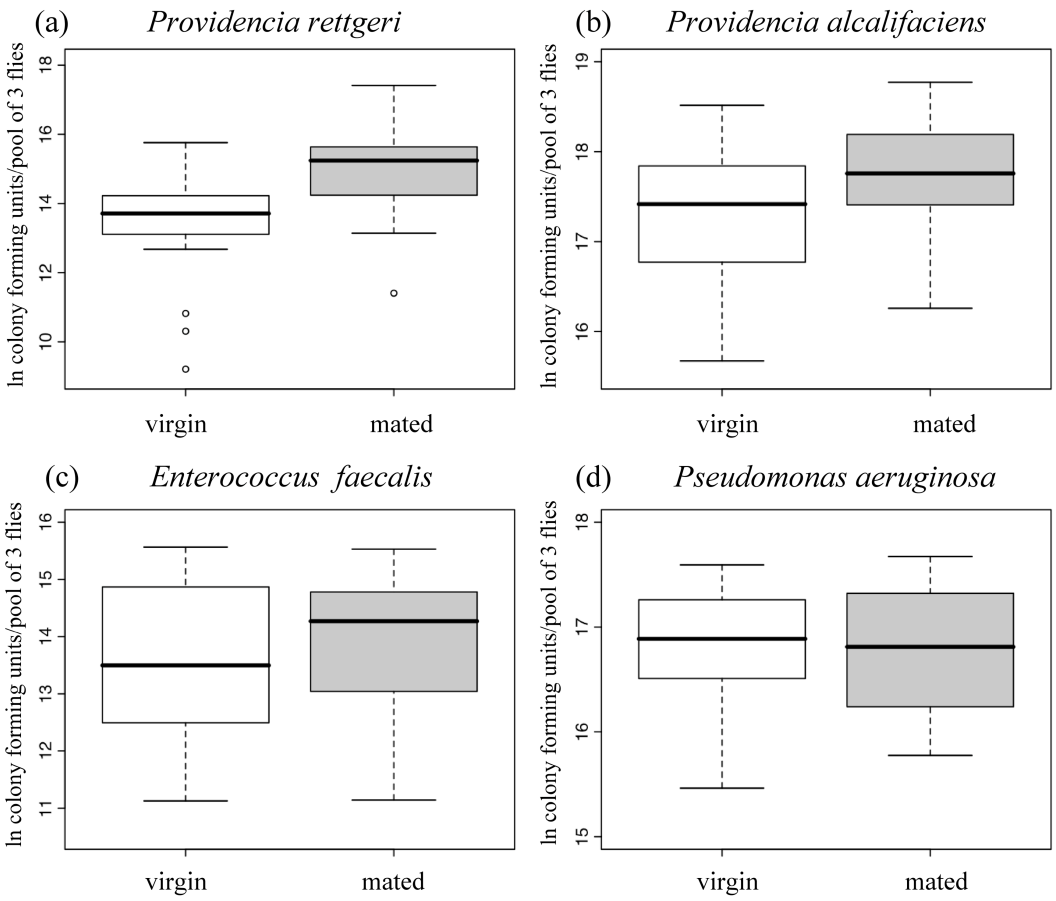

Figure 2.1. The effect of mating on female bacterial load after infection with four bacterial pathogens. Bacterial loads of wild-type (Canton S) females mated to wild-type (Canton S) males were significantly higher than those of virgin wild-type (Canton S) females after infection with (a) *Providencia rettgeri* ($F_{1,67}$ = 28.77, $p < 0.0001$) and (b) *Providencia alcalifaciens* ($F_{1,77}$ = 9.86, p = 0.0024), but not after infection with (c) *Enterococcus faecalis* ($F_{1,52}$ = 1.20, p = 0.279) or (d) *Pseudomonas aeruginosa* ($F_{1,32}$ = 0.17, p = 0.6804). We infected virgin and mated females in parallel 2-3 hours after mated females completed copulation. Total sample sizes were as follows: for *P. rettgeri,* n_{mated} = 36 and n_{virgin} = 35, for *P. alcalifaciens,* n_{mated} = 43 and n_{virgin} = 38, for *E. faecalis,* n_{mated} = 28 and n_{virgin} = 28, and for *Ps. aeruginosa,* n_{mated} = 17 and n_{virgin} = 18. Each data point consists of three pooled females, and data were collected over three replicates for each bacterial species with the exception of *Ps. aeruginosa,* for which only two replicates were collected. Uninfected controls (not shown) were sham-infected with a sterile needle and always yielded zero bacteria

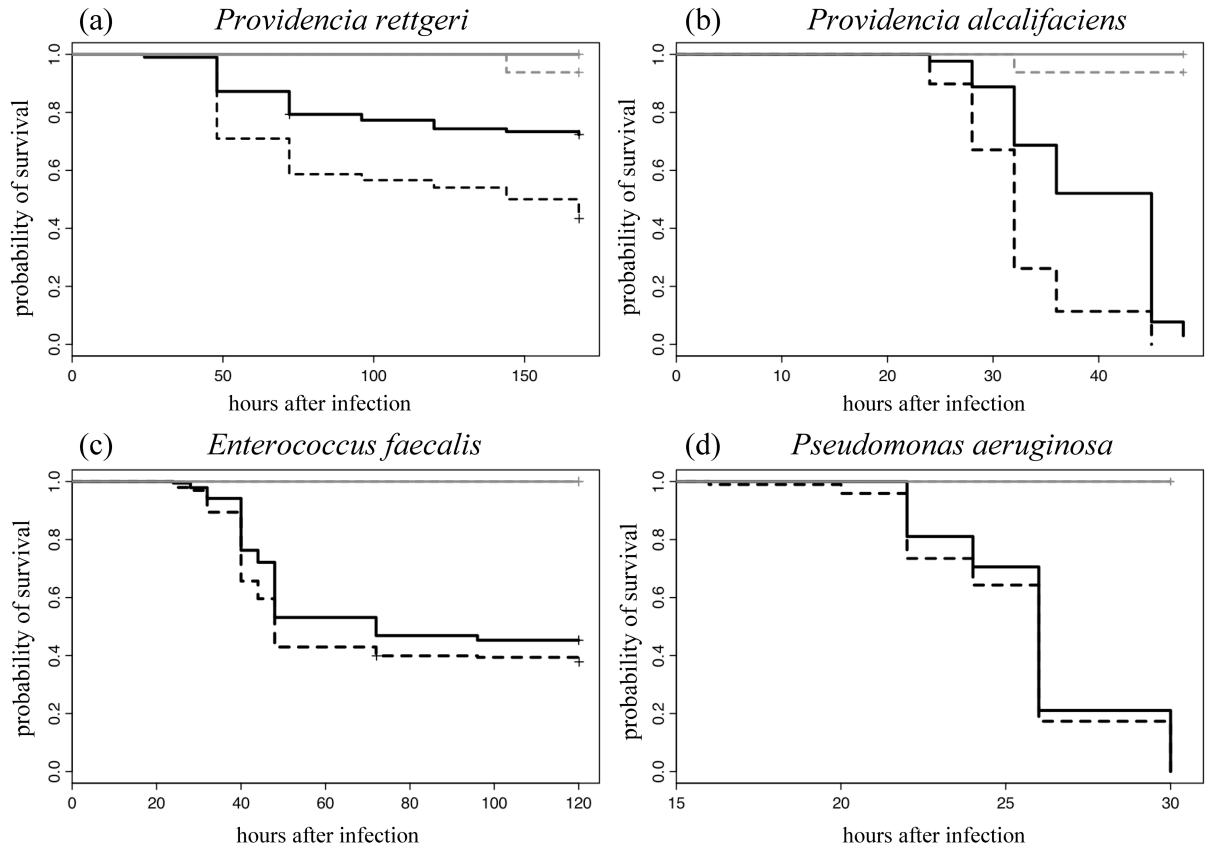

Figure 2.2. The effect of mating on female survival after infection with four bacterial pathogens. Survival over time of wild-type (Canton S) females mated to wild-type (Canton S) males was significantly lower than that of virgin wild-type (Canton S) females after infection with *P. rettgeri* (panel (a), $p < 0.0001$) and *P. alcalifaciens* (panel (b), $p < 0.0001$), but not after infection with *E. faecalis* (panel (c), $p = 0.0811$) or *Ps. aeruginosa* (panel (d), $p = 0.3466$). Survival curves were estimated using the Kaplan-Meier method. Significance values are for the effect of mating treatment in infected females and were determined by Cox regression analysis. We infected both mated and virgin females in parallel 2-3 h after mated females complete copulation. N = 44-75 infected females per mating status per replicate, and two to four replicates were performed for each survival experiment. Uninfected controls pierced with a sterile needle (lines shown in gray) showed negligible mortality for both mated (dashed gray line) and virgin (solid gray line) treatments.

quality of immune defense in female *D. melanogaster* is frequently modulated by mating, an activity that is itself clearly essential to fitness.

(b) Female, but not male, *D. melanogaster* harbor significant genetic variation for the effect of mating on immune resistance.

In order to gain insight into the evolution of the trade-off between mating and immunity, we assayed genetic variation among females for their resistance to infection after mating, genetic variation among males for their ability to alter female resistance and the degree to which the magnitude of post-mating immune depression depends on the specific male and female genotypes in mating pairs. We mated females from nine genetic lines of *D. melanogaster* to males from nine distinct genetic lines and, for all 81 pairwise crosses, assayed bacterial load after infection with *P. rettgeri* in both virgin and mated females. The entire experiment of 81 crosses was performed in duplicate, with 2-5 data points collected per cross in each replicate, where each data point is obtained from a pool of 5 females. We then performed an analysis of variance on the bacterial load data using the model in Table 2.2.

Females are highly significantly genetically variable for the degree of post-mating immune depression that they experience (mating status * female genotype, $p < 0.0001$, Table 2.2 and Figure 2.3). The bacterial load of mated females relative to virgins ranged across female genotypes from a 4.1 fold increase to essentially no change (Figure 2.3). To our surprise, however, we did not observe significant genetic variation in the ability of males to suppress female immune defense (mating status * male genotype, $p = 0.7730$, Table 2.2 and Figure 2.4). The bacterial load of mated females relative to virgins was relatively invariant across the male genotypes to which they mated, with the smallest change being a 1.4 fold increase and the largest a 2.0 fold increase. Such low levels of genetic variation among males were unexpected because

Table 2.2. Analysis of variance for effects of male and female genotype on bacterial load in mated versus virgin females. The experiment was conducted over the course of multiple days. "Experimental replicate day" refers to all days over which the experiment was conducted. "Mating status" refers to mated females versus virgins.

factor	effect type	*df*	*F value*	*p-value*
mating status	fixed	1	36.23	< 0.0001
female genotype	fixed	8	26.42	< 0.0001
male genotype	fixed	8	2.30	0.0193
experimental replicate day	random	19		
mating status * female genotype	fixed	8	4.32	< 0.0001
mating status * male genotype	fixed	8	0.61	0.7730
female genotype * male genotype	fixed	64	0.078	0.0777
mating status * female genotype * male genotype	fixed	64	1.16	0.1905
residual error		1129		

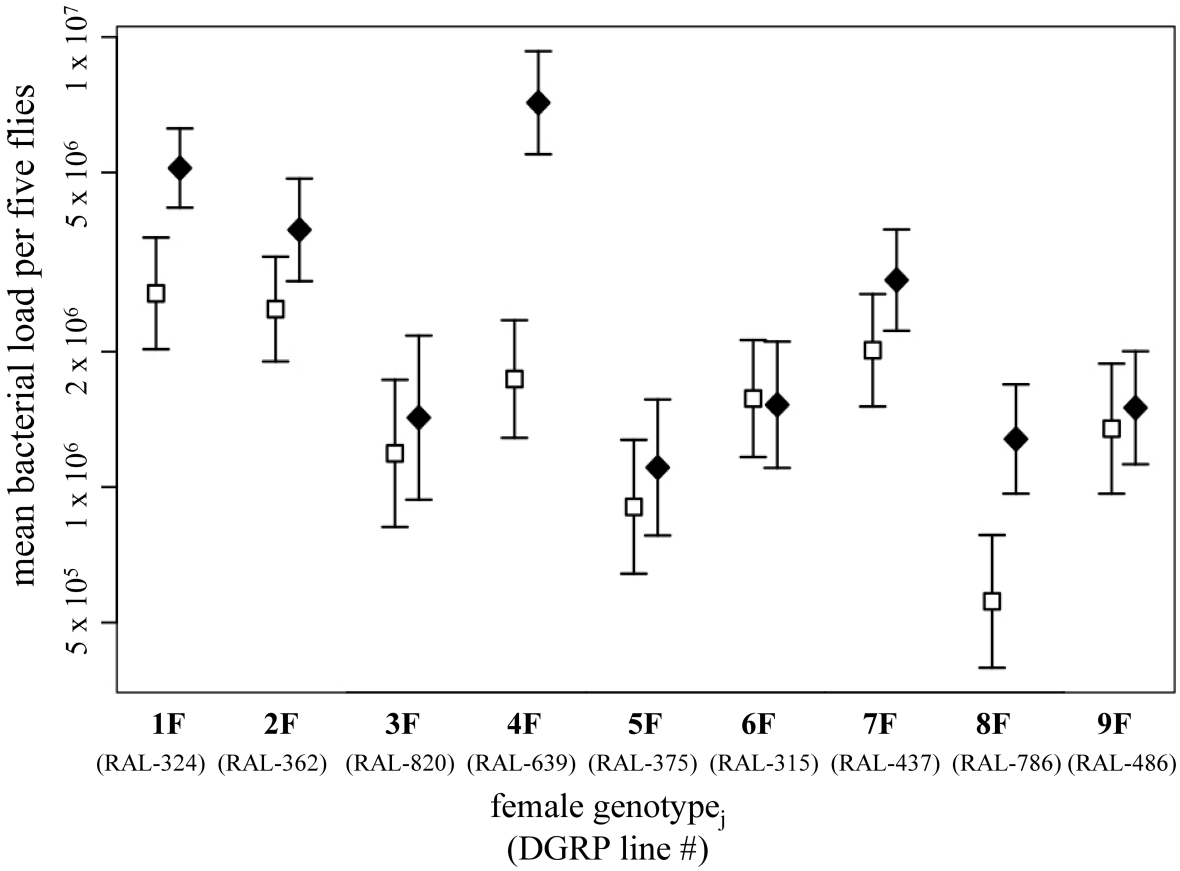

Figure 2.3. Variation in the effect of mating across female genotypes. We calculated corrected mean bacterial load for mated females (black diamonds) and virgin females (open squares) of each female genotype pooled across all male genotypes. For example, the black diamond for female 1F corresponds to the mean load sustained by 1F females after mating to males from genotypes 1M-9M, and the open square corresponds to loads sustained by virgin 1F females infected and plated alongside mated 1F females. Mean refers to geometric mean, and error bars represent a 95% confidence interval. The parenthetical numbers on the x-axis are the DGRP stock identity number.

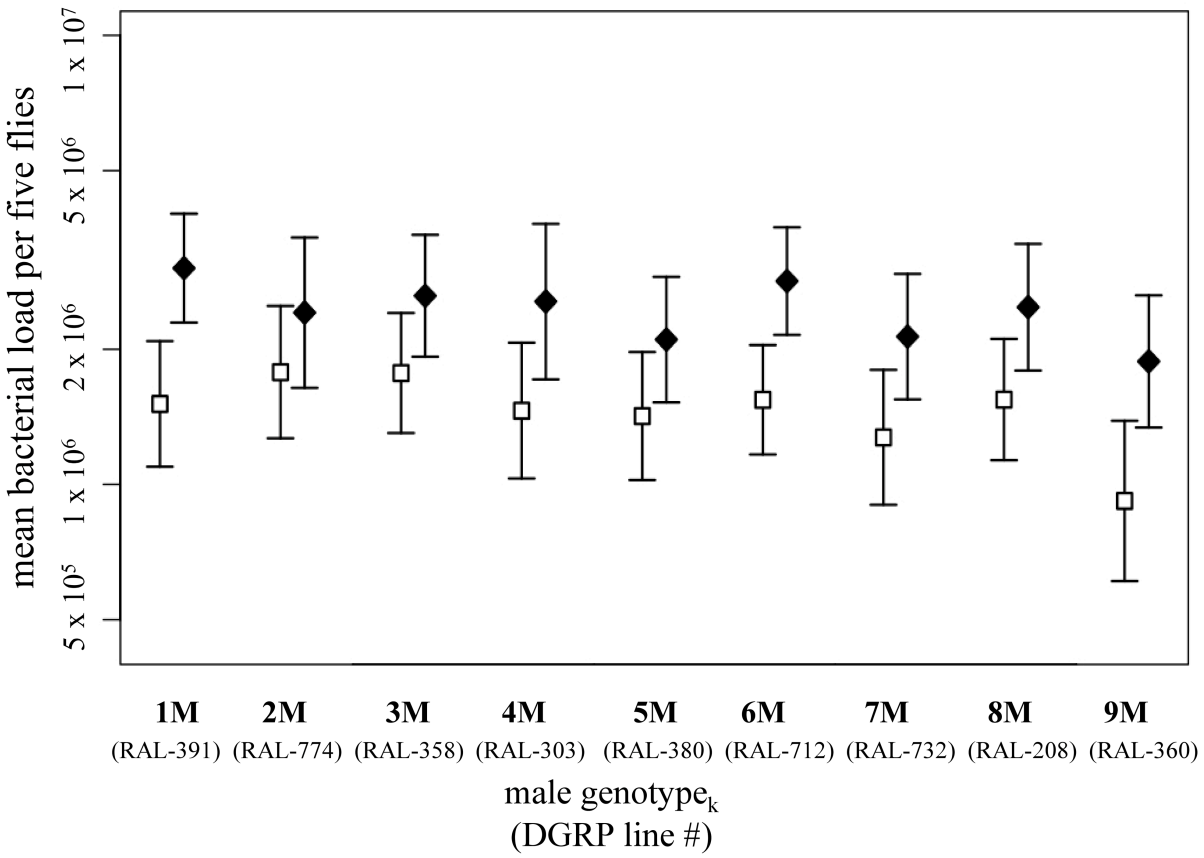

Figure 2.4. Variation in the effect of mating across male genotypes. We calculated corrected mean bacterial load for all females mated to each individual male genotype (black diamonds) and the mean bacterial load for the corresponding virgin controls from all female genotypes (open squares). For example, the black diamond for 1M corresponds to the mean of females from genotypes 1F-9F mated to 1M males, and the open square corresponds to the mean of virgin control females from lines 1F-9F infected and plated alongside the females mated to 1M males. Mean refers to geometric mean, and error bars represent a 95% confidence interval. The parenthetical numbers on the x-axis are the DGRP stock identity number.

female immune depression occurs only when the male ejaculate is intact with respect to sperm and accessory gland proteins (S. M. Short, M. F. Wolfner and B. P. Lazzaro 2010, unpublished data). Despite that, and despite the known adaptive evolution of some male ejaculate proteins, we fail to reject the null hypothesis that males are not variable for the magnitude of female immune modulation they elicit. We also found no evidence that female post-mating immune depression is determined by an interaction between the specific male and female genotypes engaged in a mating (mating status * female genotype * male genotype, $p = 0.1905$, Table 2.2), casting further doubt on any hypothesis that this trait is evolving under sexually antagonistic coevolution.

Our observation that females are highly genetically variable for the degree of post-mating immunosuppression they experience is consistent with a potential evolutionary trade-off between mating (and/or consequent reproduction) and immune defense. However, since we did not directly assay fitness in this experiment, we cannot definitively assess the possibility of an evolutionary trade-off. If such a trade-off does exist, the genetic variability we observe could reflect antagonistic pleiotropy coupled with spatial or temporal environmental variation. In this scenario, conflicting selective pressures related to immunity and reproduction could lead to maintenance of genetic variation (Gillespie & Turelli 1989; Lazzaro & Little 2009). The observation reported here and in McKean & Nunney (2005), Fedorka *et al*. (2007), Wigby *et al*. (2008) that mating induces susceptibility to some infections more than to others suggests that microbial heterogeneity might be one such example of environmental variation.

It has been hypothesized that ongoing sexual conflict could manifest in manipulation of female immune defense, such that males could potentially increase their fitness by reducing female immune defense in favor of reproduction (*e.g.* Lawniczak *et al*. 2007; Fedorka *et al*.

2007). However, the fact that we did not observe significant male genetic variation for post-mating female immunosuppression renders this hypothesis unlikely. Our data are not consistent with evolution of this trait being driven by ongoing sexual conflict.

Conclusions

In this work, we showed that female *D. melanogaster* become more susceptible to infection with two different natural bacterial pathogens after mating. Mated females sustained higher bacterial loads and lower survival compared with virgins. However, infection with two other pathogens was not more severe in mated females relative to virgins, revealing the mating effect to be pathogen-dependent. Wild females harbor substantial genetic variation for the magnitude of post-mating susceptibility they experience, but males harbor little if any genetic variability for the degree of immunosuppression they can drive. This effectively eliminates ongoing interlocus sexual conflict as a possible evolutionary scenario under which this trait could be evolving in the sampled population. It is much more likely that there is a physiological and perhaps evolutionary trade-off in females between reproduction and immune defense.

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Chapter 3.

Investigating the causes and implications of natural genetic variation for the effect of mating on immune defense in female *Drosophila melanogaster*

Abstract:

The systemic immune defense of female *Drosophila melanogaster* is reduced due to mating, suggesting a physiological trade-off between immune defense and mating or consequent reproduction. The magnitude of the physiological trade-off is genetically variable in wild flies. I was interested in determining whether this genetically variable physiological trade-off underlies an evolutionary trade-off between defense and reproduction. In order to test this, I measured immune defense, egg production and progeny production in multiple inbred lines and outbred genotypes and tested for a negative genetic correlation between immune defense and each fecundity measure. I failed to detect negative correlations, and instead found that relationships between traits were either positive or absent. I also found strong evidence for inbreeding depression in our inbred lines. These data suggest that genetic variation for the physiological trade-off is likely caused by recessive deleterious alleles and not by a straightforward evolutionary trade-off.

Introduction:

Immune defense has been shown to interact physiologically with a number of life history traits including mating (Fedorka et al., 2007; Short and Lazzaro, 2010), egg production (Fellowes et al., 1999) and longevity (DeVeale et al., 2004; Libert et al., 2006). In some cases, these interactions have been shown to have a genetic basis and to be genetically variable. For example, in a study by Ye et al. (2009), selection for increased survival after bacterial infection resulted in decreased egg viability and longevity. This result suggests not only that there is standing genetic variation for these traits, but also that there is an inverse genetic relationship between immune defense and certain important fitness traits. Consideration of genetic

interactions such as this one provides a crucial contribution to our understanding of the evolution of both immune defense and life history traits, and can provide important insights into the maintenance of genetic variation in immune system and life history genes. Investigations of this sort also have the potential to provide valuable insight into the function of organism level immunity.

Life-history traits have the potential to alter immune defense via pleiotropic effects on immune system activity, and antagonistic pleiotropy between immunity and life-history traits can result in evolutionary trade-offs, which can maintain genetic variation for the traits involved (Roff, 2002). A small number of studies have demonstrated evolutionary (genetic) trade-offs between immune defense and life-history traits in insects (reviewed in Schmid-Hempel, 2003). For example, in *D. melanogaster,* selection for increased ability to encapsulate a parasitoid wasp egg resulted in a concordant decrease in larval foraging ability (Kraaijeveld and Godfray, 1997) and, as mentioned above, selection for increased survival after infection by a bacterial pathogen resulted in reduced longevity and egg viability (Ye et al., 2009). Additionally, McKean et al. (2008) showed a negative genetic correlation between bacterial load levels after infection and fecundity over multiple days in female *D. melanogaster*.

The majority of studies investigating relationships between immune defense and life-history traits in insects, however, have focused on physiological trade-offs, which are distinct from evolutionary trade-offs in that they result from individual-level "use costs," not population-level "genetic costs." (Stearns, 1989; Schmid-Hempel, 2003). In the case of a physiological trade-off with immune defense and reproduction, for example, the cost of using the immune system to fight infection would manifest as a decrease in reproductive output. This may be because the two traits are competing for the same pool of resources (Sheldon and Verhulst, 1996;

Flatt, 2011). Genetically variable physiological trade-offs can underlie evolutionary trade-offs, but physiological trade-offs that are not genetically variable can also manifest within individuals and have important implications for the function of the traits involved (Flatt, 2011).

Physiological trade-offs are often revealed as a response to a treatment, and many studies in various species of insects have demonstrated the presence of physiological trade-offs between immune defense and life-history traits (reviewed in Schmid-Hempel, 2003). For example, in damselflies, increased oviposition results in a decrease in encapsulation ability (Siva-Jothy et al., 1998), and mating in crickets reduces multiple components of immune system activity (Fedorka et al., 2004). In beetles, mating results in reduced phenoloxidase activity (Rolff and Siva-Jothy, 2002), and infection challenge causes decreased longevity (Armitage et al., 2003). In *D. melanogaster,* successful encapsulation of a parasitoid wasp egg results in reduced adult female fecundity (Fellowes et al., 1999), and higher rates of courtship and mating result in reduced ability to clear non-pathogenic bacteria in males (McKean and Nunney, 2001). We and others have shown that females experience a significant reduction due to mating in their ability to resist and survive (Fedorka et al., 2007; Short and Lazzaro, 2010) infection by pathogenic bacteria, suggesting a physiological cost of mating in females. I have also demonstrated that females are genetically variable for this physiological trade-off (Short and Lazzaro, 2010). In this study, I aimed to determine whether this physiological trade-off does indeed underlie an evolutionary trade-off between immune defense and reproduction. In order to test this, I examined whether change in immune defense due to mating correlated with either egg production or fecundity. I found no evidence of a negative genetic correlation in either inbred or outbred female genotypes. Implications and potential explanations for these results are discussed.

Materials and Methods:

Fly lines and maintenance: Potential trade-offs were measured in separate experiments using either inbred or outbred genotypes. For the experiment using inbred lines, I used 13 lines of the *Drosophila* Genetic Reference Panel (Mackay et al., 2012). Females were taken from RAL-437, RAL-362, RAL-820, RAL-786, RAL-359, RAL-716, RAL-639, RAL-142, RAL-315, RAL-324, RAL-486 and RAL-375. These females were mated to males from a distinct line, RAL-358. For the experiment using outbred flies, females from these same 12 lines were outcrossed to Canton-S males and F1 daughters were used in the experiment. To generate F1 males for use in the experiment, females from RAL-358 were outcrossed to RAL-774 males. For all experiments, flies were reared on standard Cornell *Drosophila* media (8.3% glucose, 8.3% Brewer's yeast, and 1% agar, plus 0.04% phosphoric acid and 0.4% propionic acid added to inhibit microbial growth in the food) and were maintained at 24ºC on a 12 hr light/dark cycle.

Density control and virgin collection: To generate density-controlled females and males for use in the inbred line experiment, approximately 20 female parents and approximately 10 male parents from the lines listed above were combined in a fresh food vial and allowed to lay eggs for 4-24 hours, until approximately 100 eggs had been laid, at which point the parents were removed from the vials. To generate density-controlled F1females and males for the outbred experiment, approximately 8 female parents from the inbred DGRP lines listed above and approximately 5 male Canton S parents were combined in a fresh food vial and allowed to lay eggs for approximately 24 hours, at which point the parents were removed from the vials. Inbred and outbred progeny from these vials were used for our mating experiments. These density-control methods resulted in a mean density of 121 pupae (SD = 49) per vial for inbred lines and 87 pupae (SD = 37) per vial for outbred genotypes. Three to four days before all experiments,

virgin males and females were collected from the density-controlled vials and housed in groups of 30 (for males) and 20 (for females) until use.

Mating setup: The day preceding each experiment, virgin females were lightly anesthetized on CO_2 and deposited into individual vials. They were then randomly allocated to "virgin" or "mated" treatments. The following day, individual male virgins were aspirated without anesthesia into each vial containing a female assigned to be mated. All matings for a single cross or line were set up together, and lines and crosses were randomly ordered within each replicate using the sample( ) command in R (R Foundation for Statistical Computing, Vienna, Austria). All matings were observed every five minutes until completion and those pairs that remained in copula for fewer than ten minutes were discarded. Within approximately 30 minutes (max 1.5 hours) after mating, mated females were removed from the presence of males to prevent additional courting or copulation attempts.

Infection and Survival: All infections were performed at 2.5 hours (±0.5 hrs) after mating, and virgin females from the same genotype were infected in parallel with their mated counterparts. To infect each female, I pierced the thorax with a 0.15 mm anodized steel needle (FST) dipped in dilute bacterial culture. The bacteria I used for infection was *Providencia rettgeri*, which was cultured overnight at 37°C and diluted in sterile LB to A_{600}=1.0 for infections. This method introduced an average of 2.5×10^3 bacteria to each fly. In parallel with infections for each experiment, wounding control females were pierced with a sterile needle. After infection or sterile wounding, females were placed in individual vials with *ad libidum* access to food to recover. Those that did not recover, *i.e.* were dead or moribund at two hours after infection, were discarded. Survival was then recorded daily for five days with the exception of one of the three replicates of the inbred dataset, for which I only observed survival for four

days. Over this time period, fewer than 5% of inbred and outbred mated females failed to lay fertile eggs. I interpreted these instances to result from unsuccessful copulations and did not include these females in our analysis. Total mortality of sterile needle controls was negligibly low over the 5 day period of all of the experiments (inbred: 7 deaths/183 control flies, outbred: 4 deaths/317 control flies).

Egg laying and progeny production: Females that were used for egg counting assays were not infected. After mating, females were separated from males and put into individual vials containing fresh media. Virgin controls were put on fresh food in parallel with mated females from the same genotype. Every day for five days, mated and virgin females were transferred to new vials and the number of eggs they laid in the previous 24 hours were counted. From days 5-10 post-mating, mated females were left in the same vial and eggs were not counted. Eggs from all mated vials, including the final vial from days 5-10 post-mating were allowed to hatch and pupate, and adult progeny that successfully eclosed were counted for days 1-10 post-mating. Mated females that did not lay fertile eggs were considered to have mated unsuccessfully and were excluded from analysis.

While collecting fecundity data for the inbred lines, we noticed that RAL-437 females showed an exceptional pattern of egg-laying. Most females from this line laid eggs for the first 24 hours after mating and then few or no eggs after this day. This is very different from the other lines (which continue to produce eggs for many days after mating) and suggests that females from this line may have a mutation that eliminates their long term post-mating response (Ravi Ram and Wolfner, 2007). This warrants further investigation, but since the phenotype of this line seems to be driven by a strong and presumably deleterious recessive mutation, we eliminated the inbred line RAL-437 from subsequent analysis. The heterozygous genome from this line (cross

RAL-437 x CS) shows no such aberrant egg-laying pattern and was retained in analysis of data from the outbred genotypes.

Statistical analysis:

We tested the effect of line on difference in overall survival between mating statuses. To test this, we subtracted the final proportion of control virgin females alive at the end of each replicate from the final proportion of mated females still alive for that replicate. We then used these values as our response variable and performed a one-way ANOVA using genotype as the predictive factor. We also assessed the effect of mating status and inbred line/outbred genotype on survival over time after infection using Cox Regression analysis in R (Therneau, 2012). Event data (where an "event" = death) were recorded for flies from each mating status and each genotype and flies that were still alive at the end of the observation period were treated as censored data. Mating treatment, genotype and replicate were included as factors in all regression analyses, as was a mating treatment * genotype interaction term, which tests for differential survival due to mating across inbred lines or outbred genotypes.

We assessed the effect of mating status and inbred line/outbred genotype on total egg production or progeny production by performing an analysis of variance in R (R Foundation for Statistical Computing, Vienna, Austria). For total egg production, we included mating status and inbred line/outbred genotype as factors in the model as well as an interaction between these terms to assess differential effects of mating on egg-laying levels across lines/genotypes. For total progeny production at 120 hours or 240 hours post-mating, we assessed only the effect of inbred line/outbred genotype on the response variable.

To assess relatedness between immune defense and fecundity phenotypes, we calculated Pearson's correlation coefficients and tests for significance using the cor.test() command in R (R Foundation for Statistical Computing, Vienna, Austria)

Results:

Analysis of flies from inbred lines

I measured survival after infection in mated females and virgin controls from 12 inbred lines established from a wild population in Raleigh, N.C (Table 3.1). As before (Short and Lazzaro, 2010), I found that mating causes a significant reduction in survivorship of infection (Table 3.1 and Table 3.2, $p = 0.028$). As will be explained in more detail below, in order to assess whether immune defense correlates with fecundity, I determined the proportion of mated females that survived to the end of the observation period relative to virgin controls for each replicate of the experiment (Table 3.1). Using these values, which represent the overall change in survival due to mating, I performed an analysis of variance and found significant variation among lines (ANOVA for the effect of line on the difference in final survival between mating statuses; $F_{10,14} = 2.64$, $p = 0.048$, Figure 3.1). This indicates significant genetic variation for the effect of mating on overall survival of infection. This is consistent with data I have previously published showing significant genetic variation for the effect of mating on immune defense when measured as bacterial load (Short and Lazzaro, 2010).

I also used a Cox regression analysis to test for genetic variation in the effect of mating on survival over time. This analysis evaluates whether the shapes of the survival curves of virgin females vary from those of mated females. I found that survival over time after infection varied significantly across inbred lines regardless of female mating status ($p = 4.10 \times 10^{-10}$, Table 3.2).

Table 3.1: Survival after infection, egg production and progeny production for each inbred line

InbredLine	Virgin female survival (n)	Mated female survival (n)	Virgin avg. total eggs, days 1-5 (std. error, n)	Mated avg. total eggs, days 1-5 (std. error, n)	Avg. total progeny, days 1-5 (std. err)*	Avg. total progeny, days 1-10 (std. err)*
RAL-437	0.60 (25)	0.81 (26)	11.3 (4.6, n=10)	27.1 (9.5, n=9)	21.2 (7.5)	39.8 (13.9)
RAL-362	0.59 (32)	0.66 (35)	8.6 (4.5, n=10)	91.8 (9.2, n=9	70.4 (10.1)	84.3 (13.8)
RAL-820	0.71 (34)	0.41 (27)	0.0 (0.0, n=3)	60.0 (36.9, n=3)	52.3 (31.5)	80.0 (37.7)
RAL-786	0.57 (28)	0.50 (32)	27.8 (9.0, n=9)	100.1 (15.2, n=7)	59.4 (10.3)	83.7 (15.4)
RAL-359	0.30 (20)	0.30 (20)	92.0 (11.2, n=10)	127.2 (8.2, n=6)	65.5 (16.4)	68.5 (17.0)
RAL-716	0.70 (20)	0.58 (19)	46.7 (8.9, n=10)	112.7 (12.9, n=10)	93.5 (12.0)	136.3 (17.4)
RAL-639	0.42 (19)	0.37 (19)	59.3 (7.0, n=9)	90.6 (10.0, n=7)	39.0 (7.1)	50.7 (9.9)
RAL-142	0.75 (20)	0.61 (18)	23.7 (9.2, n=9)	127.7 (12.3, n=9)	48.7 (12.2)	70.6 (17.2)
RAL-315	0.73 (15)	0.13 (8)	24.4 (9.1, n=5)	47.9 (10.7, n=7)	31.7 (9.1)	46.7 (12.3)
RAL-324	0.72 (18)	0.71 (14)	91.1 (8.6, n=10)	108.3 (8.4, n=8)	78.3 (11.0)	102.8 (15.2)
RAL-486	0.37 (19)	0.45 (20)	13.0 (6.5, n=8)	95.3 (10.3, n=9)	56.7 (8.3)	75.8 (9.4)
RAL-375	1.00 (20)	0.95 (20)	57.8 (9.5, n=9)	126.6 (7.0, n=9)	106.4 (4.3)	162.2 (7.5)

*Sample size for progeny production is the same as that used for mated female egg production measurements.

Table 3.2: Analysis of deviance for Cox regression analysis assessing the effect of mating status and line on survival after infection in inbred lines

	Model log-likelihood	Chi square	d.f.	p-value
Null	-1178.1			
Experimental rep	-1174.1	7.99	2	0.01837
Mating status	-1171.7	4.81	1	0.02826
Inbred line	-1139.2	64.97	10	4.10×10^{-10}
Mating status * Line	-1132.8	12.89	10	0.22998

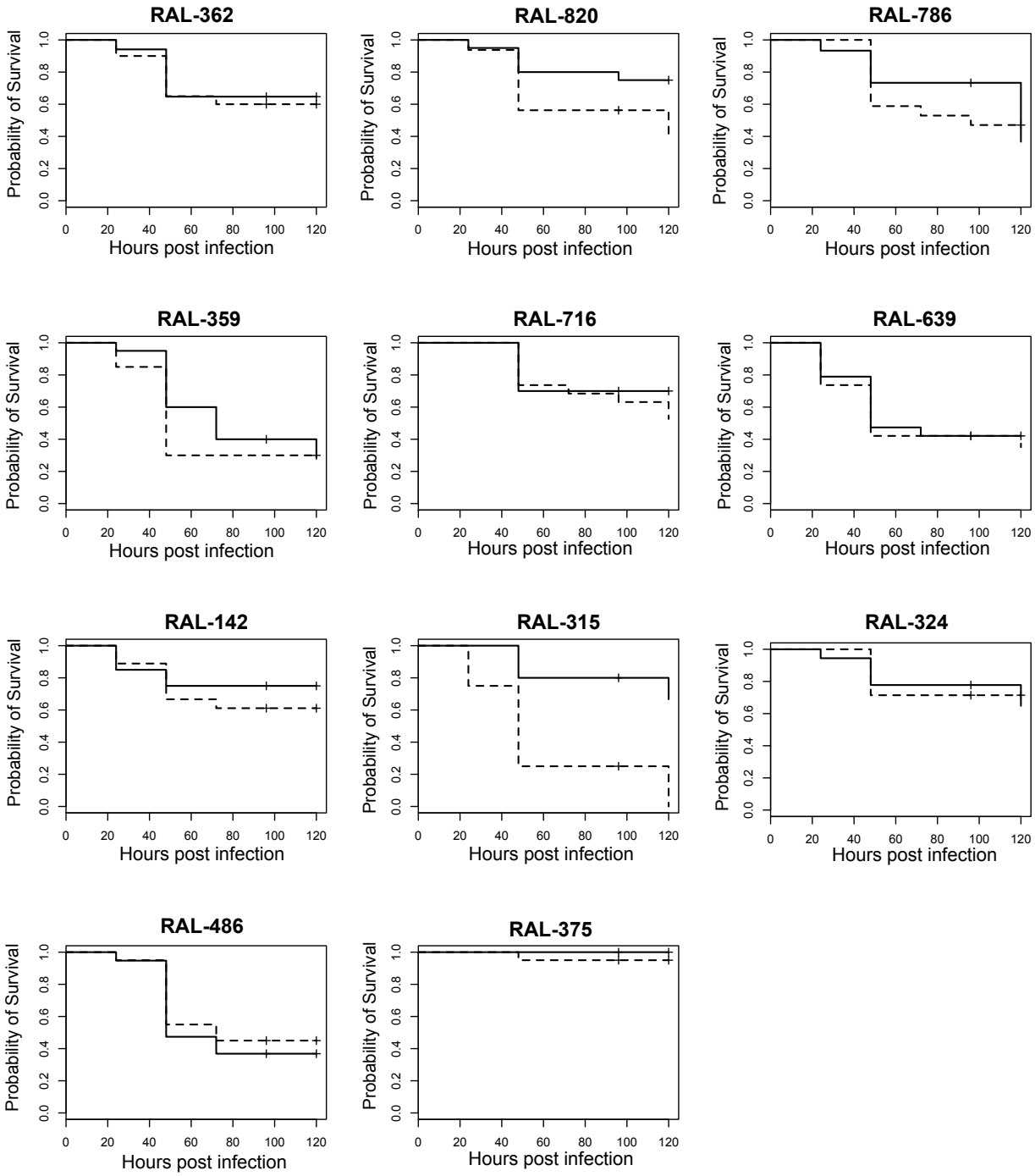

Figure 3.1: Survival of infection by mated and virgin females from eleven inbred lines. Mated females (dashed lines) from each of eleven inbred lines were infected approximately 2.5 hours after the completion of copulation. Virgin controls (solid lines) were infected in parallel for each line.

I failed to detect a significant interaction between mating status and line, suggesting that, at any given time post-infection, the rate at which mated females succumb to infection relative to virgins does not vary among lines ($p = 0.230$, Table 3.2, Figure 3.1). This was unexpected, considering I detected significant genetic variation for the effect of mating on final survival. It is unclear why these analysis methods gave different results. It is possible that there is more variability in the shape of the survival curves than in the overall rates of survival and that the Cox regression is underpowered relative to the experimental variability in mortality rates across replicates.

I measured egg production over the first five days post-mating and adult progeny production over the first ten days post-mating in uninfected females from these same inbred lines (Table 3.1). *D. melanogaster* females tend to lay low numbers of unfertile eggs as virgins but many eggs post-mating, so mating status was a highly significant predictor of egg production, ($p < 1.0 \times 10^{-15}$, Table 3.3). The genetic lines were highly significantly variable in their total egg production ($p = 1.66 \times 10^{-14}$, Table 3.3). Line was also a significant predictor of total progeny production after five days post-mating ($F_{10,73} = 4.43$, $p = 7.12 \times 10^{-5}$) and after ten days post-mating ($F_{10,73} = 6.05$, $p = 1.24 \times 10^{-6}$).

In order to test for a correlation between immune defense and fecundity, I first expressed the effect of mating on immune defense in each inbred line as the proportion of total mated females that survived infection minus the proportion of total virgins that survived. A higher value therefore represents higher post-mating immune defense while a lower value represents lower post-mating immune defense. I then assessed whether post mating immune defense correlated with our measurements of fertility and fecundity in the inbred lines (Figure 3.2). I found a significantly positive correlation between post-infection survival of mated females

Table 3.3: Analysis of variance assessing the effect of mating status and line on egg production in inbred lines

Factor	d.f.	Sum of squares	F-value	p-value
Mating status	1	143967	171.79	$< 1 \times 10^{-15}$
Inbred line	10	95678	11.42	1.66×10^{-14}
Mating status * Line	10	32116	3.83	0.000121
Residual	154	129055		

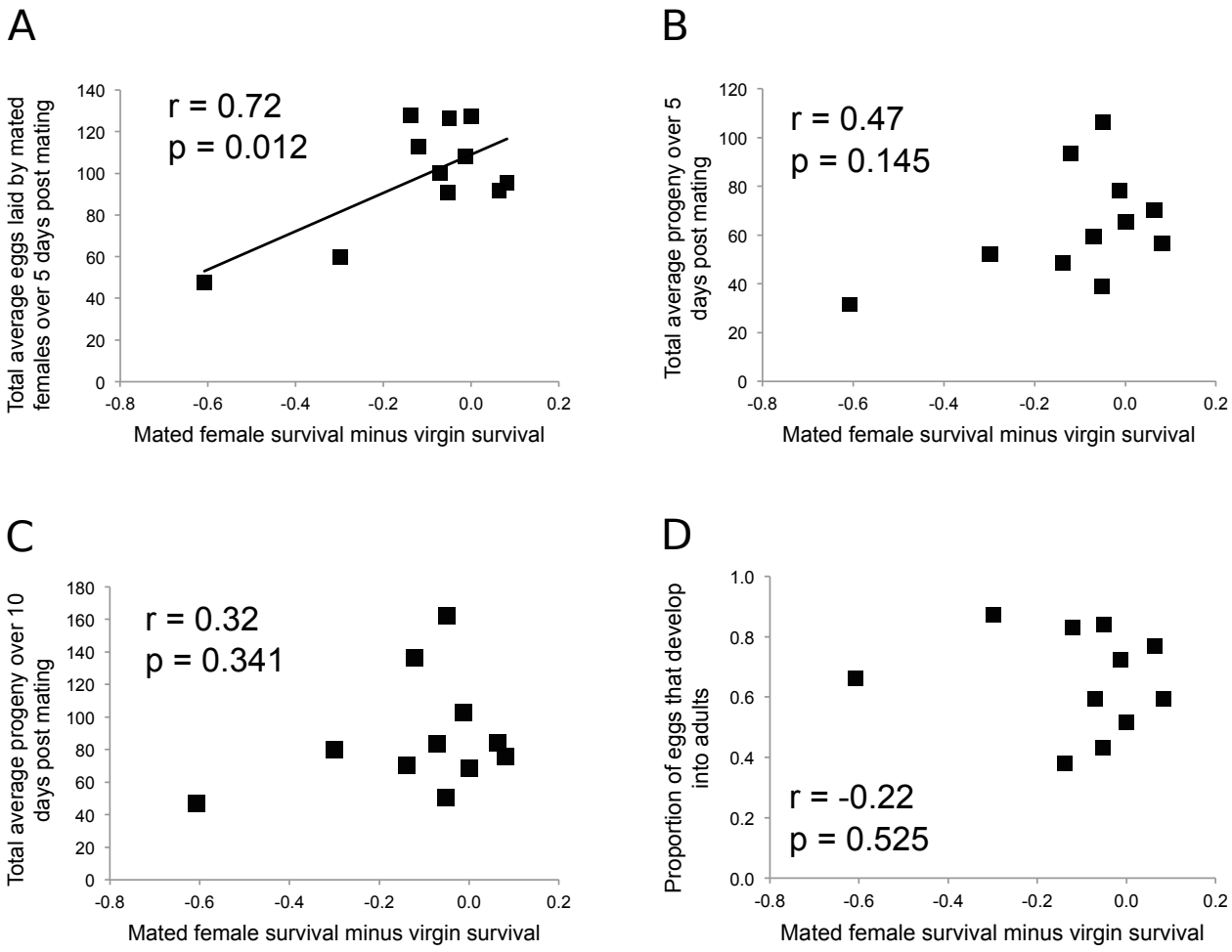

Figure 3.2: The relationships between post-mating immune defense and fitness-related traits in inbred lines. Post-mating immune defense is expressed as the proportion of mated females that survived infection minus the proportion of virgin control females that survived infection in the same inbred line. Higher values therefore correspond to higher post-mating immune defense and lower values to lower defense. The strength of relationships between traits was evaluated by determining Pearson's correlation coefficients for each comparison.

relative to virgins and total number of eggs produced by uninfected mated females (r = 0.72, p = 0.012, Figure 3.2A), meaning that inbred lines with high post-mating immune defense also laid more eggs when uninfected. Survival of infection of mated relative to virgin females was not significantly correlated with progeny production at either five or ten days after mating (Figure 3.2B, C).

For the first five days after mating, we collected data for both the total average egg production and the total number of adult progeny that successfully developed from the counted eggs. Egg survivorship was not significantly correlated with mated female immune defense (r = -0.22, p = 0.525, Figure 3.2D), but it was weakly negative, which is in contrast to the positive correlation values in Figures 3.2A-C. It is possible that this weak negative relationship, though not significant, explains why we detected a significant positive correlation between mated female immune defense and egg production but not mated female immune defense and progeny production. Females from inbred lines with high post-mating immune defense laid large numbers of eggs, but those eggs may have had relatively low survivorship. Therefore, females with high immune defense had eventual progeny numbers similar to those of females with low immune defense, resulting in no relationship between immune defense and ultimate progeny production.

Analysis of outbred flies

To control for potential effects of inbreeding on the correlations between immunity and reproductive life history traits, I outcrossed the twelve inbred lines used in the previous experiment to Canton S and used the F1 female progeny in an experiment analogous to that described above. F1 females within these crosses are genetically identical, and vary from females from other crosses by only the maternal complement of their genome. For each outbred

genotype, I measured post-infection survival of mated and virgin females as well as egg and progeny production (Table 3.4). I found no effect on overall survival and each line showed similar proportions of overall mortality in the mated and virgin treatments (ANOVA for the effect of line on the difference in final survival between mating statuses; $F_{11,24} = 0.664$, $p = 0.757$, Figure 3.3). Using a Cox regression analysis, mating had a highly significant effect on survival of infection ($p = 1.61 \times 10^{-7}$, Table 3.5), with mated females demonstrating generally lower survival than virgin controls regardless of genotype (Table 3.4, Figure 3.3). Survival did not significantly vary among outbred genotypes ($p = 0.085$, Table 3.5, Figure 3.3). I also failed to detect an interaction between mating status and outbred genotype on survival of infection, indicating that the magnitude of the effect of mating on survival of infection is similar over time across all outbred genotypes ($p = 0.746$, Table 3.5, Figure 3.3).

Total egg production increased significantly after mating ($p < 1.0 \times 10^{-15}$, Table 3.6) and varied significantly across outbred genotypes, ($p = 1.51 \times 10^{-15}$, Table 3.6). I detected genetic variation among outbred females for total progeny production after five days post-mating ($F_{11,92} = 2.28$ $p = 0.016$) but not after ten days post-mating ($F_{11,91} = 1.34$ $p = 0.216$) .

As in the inbred analysis, I determined the effect of mating on immune defense in each outbred genotype by expressing mated female survival of infection relative to virgin survival. I then tested for correlations between relative mated female immune defense and egg production or progeny production, but found no evidence of a correlation between post-mating immune defense and either of the reproductive traits (Figure 3.4).

Table 3.4: Survival after infection, egg production and progeny production for females derived from twelve outbred crosses

Outbred genotype (Female x Male)	Virgin female survival (n)	Mated female survival (n)	Virgin avg. total eggs, days 1-5 (std. error, n)	Mated avg. total eggs, days 1-5 (std. error, n)	Avg. total progeny, days 1-5 (std. err)*	Avg. total progeny, days 1-10 (std. err)*
RAL-437 x CS	0.89 (28)	0.71 (29)	37.8 (8.7, n=9)	196.9 (8.5, n=9)	194.2 (9.0)	333.9 (8.0)
RAL-362 x CS	1.00 (28)	0.87 (30)	0.0 (0.0, n=9)	163.5 (18.8, n=8)	158.9 (19.9)	299.7 (39.9)
RAL-820 x CS	0.97 (29)	0.76 (29)	23.1 (9.3, n=10)	183.7 (16.3, n=10)	175.3 (16.3)	288.3 (22.0)
RAL-786 x CS	0.93 (28)	0.74 (27)	51.8 (9.0, n=10)	233.1 (11.6, n=7)	213.9 (11.4)	366.2 (15.3)
RAL-359 x CS	0.93 (29)	0.86 (29)	70.9 (12.9, n=7)	204.8 (5.1, n=10)	199.4 (5.7)	327.0 (11.1)
RAL-716 x CS	0.93 (27)	0.83 (29)	83.6 (10.8, n=8)	212.6 (7.4, n=8)	203.5 (6.8)	336.8 (11.5)
RAL-639 x CS	0.93 (27)	0.68 (28)	68.3 (12.3, n=10)	206.4 (6.8, n=10)	201.1 (6.5)	305.9 (10.8)
RAL-142 x CS	0.90 (29)	0.76 (29)	41.1 (11.9, n=10)	201.0 (5.8, n=9)	186.8 (7.8)	302.6 (16.3)
RAL-315 x CS	1.00 (22)	0.86 (22)	7.1 (3.6, n=9)	197.8 (7.6, n=6)	190.2 (8.5)	290.2 (9.5)
RAL-324 x CS	0.90 (30)	0.76 (25)	5.2 (2.5, n=10)	176.0 (8.0, n=10)	175.3 (8.6)	311.0 (12.7)
RAL-486 x CS	0.93 (30)	0.96 (26)	12.0 (4.6, n=9)	202.4 (14.7, n=8)	198.4 (14.3)	327.9 (27.3)
RAL-375 x CS	0.97 (30)	0.90 (29)	71.7 (5.9, n=10)	220.7 (9.9, n=9)	219.8 (11.7)	335.6 (15.7)

*Sample size for progeny production is the same as that used for mated female egg production measurements.

Table 3.5: Analysis of deviance for Cox regression analysis assessing the effect of mating status and genotype on survival after infection in outbred genotypes

	Model log-likelihood	Chi square	d.f.	p-value
Null	-547.28			
Experimental rep	-546.02	2.52	2	0.28315
Mating status	-532.29	27.45	1	1.61×10^{-7}
Outbred cross	-523.37	17.84	11	0.08536
Mating status * cross	-519.56	7.63	11	0.74576

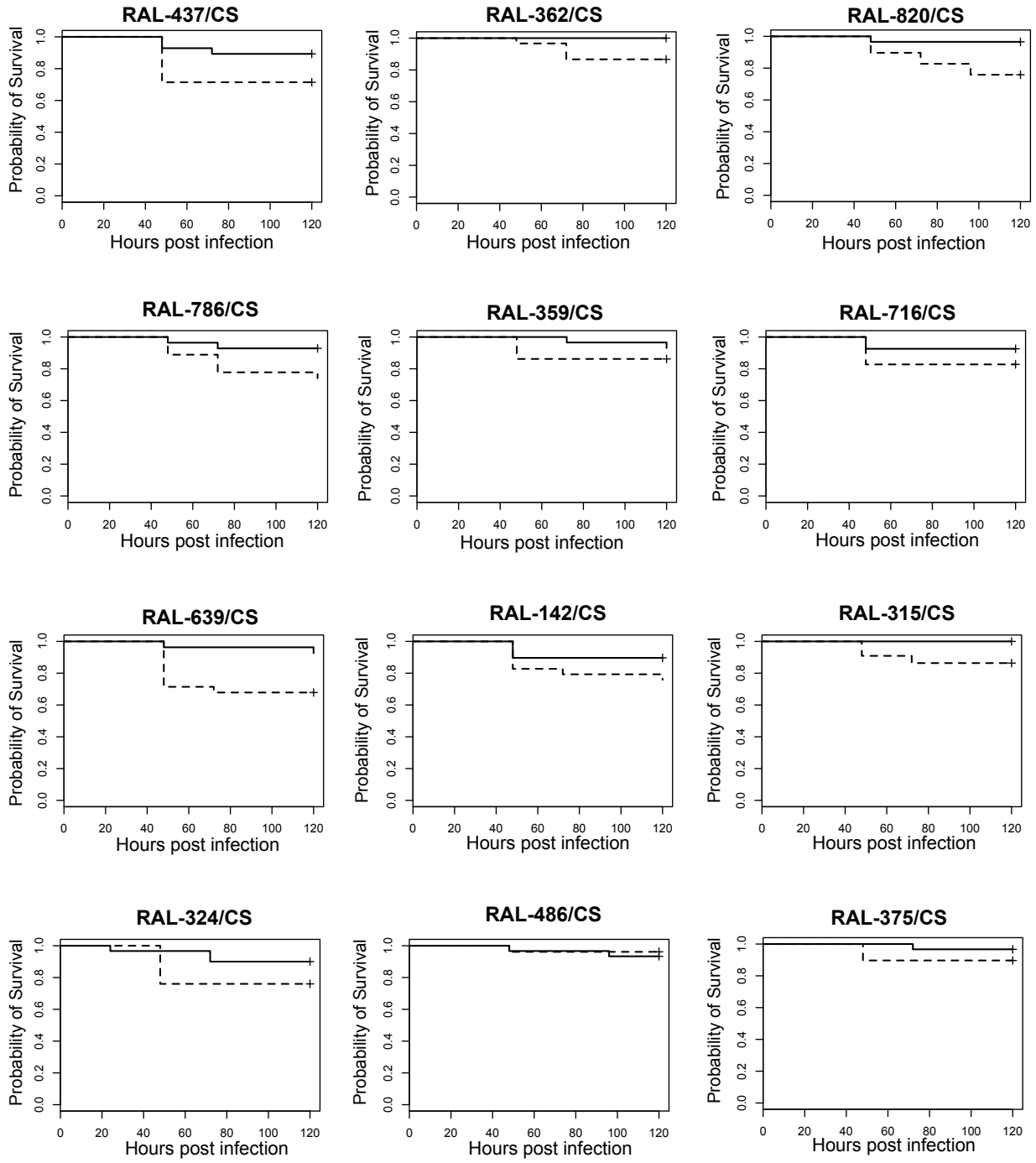

Figure 3.3: Survival of infection by mated and virgin females from twelve outbred genotypes. Mated females (dashed lines) from each of twelve outbred genotypes were infected approximately 2.5 hours after the completion of copulation. Virgin controls (solid lines) were infected in parallel for each genotype. The outbred females were generated by outcrossing the inbred lines used in Figure 3.2 to Canton S.

Table 3.6: Analysis of variance assessing the effect of mating status and genotype on egg production in outbred genotypes

Factor	d.f.	Sum of squares	F-value	p-value
Mating status	1	1385214	1655.5	$< 1 \times 10^{-15}$
Outbred cross	11	100549	10.92	1.51×10^{-15}
Mating status * cross	11	19436	2.11	0.02125
Residual	191	159821		

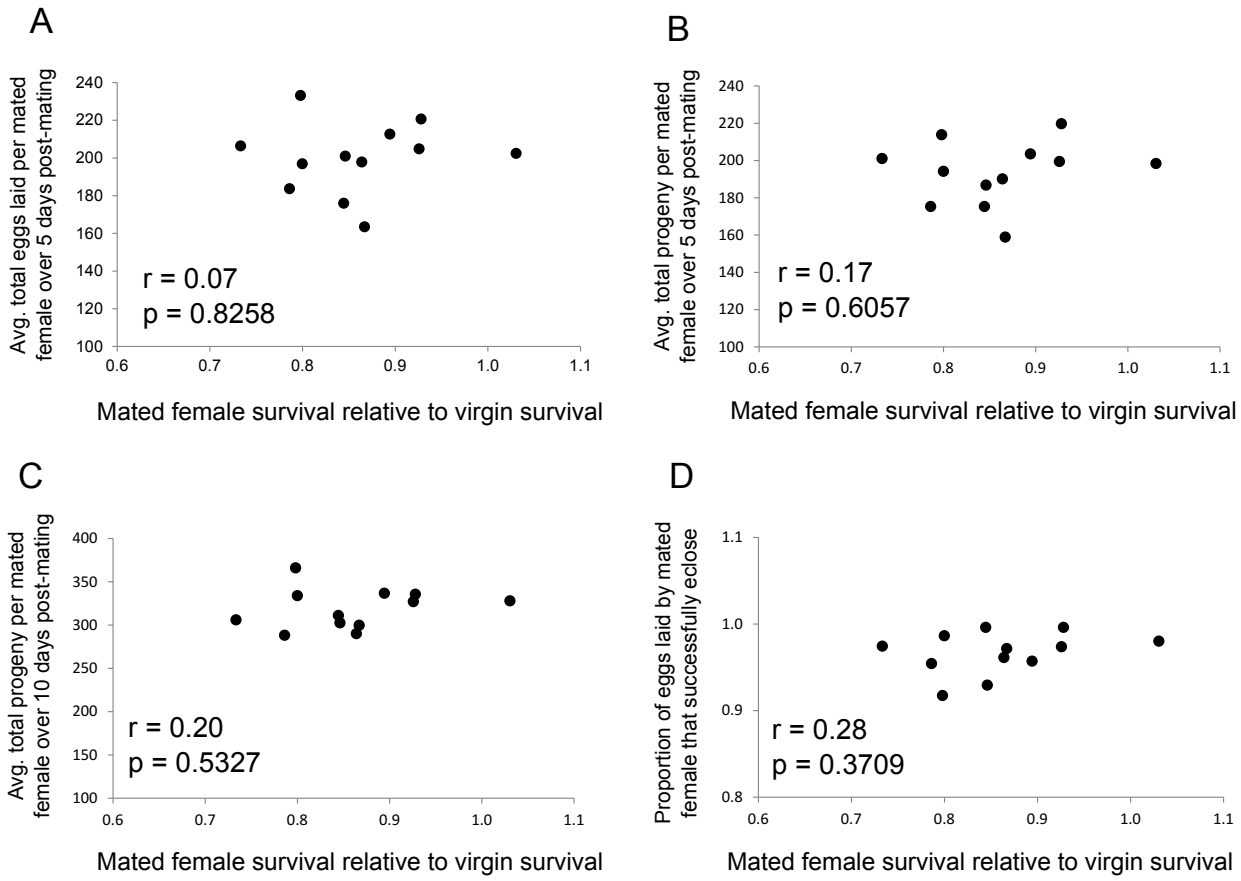

Figure 3.4: The relationships between post-mating immune defense and fitness-related traits in outbred genotypes. Post-mating immune defense is expressed as the proportion of mated females that survived infection divided by the proportion of virgin females that survived infection in the same outbred cross. Higher values therefore correspond to higher post-mating immune defense and lower values to lower defense. The strength of relationships between traits was evaluated by determining Pearson's correlation coefficients for each comparison.

Discussion

In this study, I was interested in determining whether females with reduced post-mating survival of infection demonstrated increased performance of fitness traits. I tested this by measuring the correlation of post-mating immune defense with egg/progeny production in uninfected flies across a panel of inbred lines and outbred genotypes. Among inbred lines, I detected a significant positive correlation between post-mating immune defense and egg production, where inbred lines with high survival of infection after mating laid high numbers of eggs. Among outbred genotypes, I detected no correlations among any of the measured traits. I found that outbred lines had a generally higher level of performance for all traits measured, suggesting measurable inbreeding depression within the inbred lines. I found evidence for genetic variation for the effect of mating on overall survival in inbred lines, but not for survival over time. The presence of genetic variation for the effect of mating on survival is consistent with my previous observation that there is a high level of genetic variation among a subset of these same lines for the effect of mating on bacterial load levels after infection (Short and Lazzaro, 2010). I was surprised that this same result was not observed using a Cox regression. The experimental design in Short and Lazzaro (2010) provided very high power to test for variation among female genotypes, and it is possible that I lack sufficient power in this experiment to detect variation for the effect of mating on survival over time. Regardless, the results of the analysis of variance I performed on final survival differences between mating statuses suggests the presence of significant genetic variation.

These data do not provide evidence for an evolutionary trade-off. Rather, the observed inbreeding depression suggests that the genetic variation I detected in the inbred lines is due to deleterious recessive mutations that segregate at low frequency in the natural source population

and become homozygous in inbred lines. These deleterious mutations may act to reduce the overall ability of a genotype to acquire resources, and variation in acquisition of resources can result in positive genetic correlations between traits (Houle, 1991), which may explain the positive correlation I observed between post-mating immune defense and egg production in the inbred lines. Alternatively, deleterious alleles may cause direct damage to the traits I measured, rather than indirectly affecting them via resource acquisition. In this case, the positive correlation I observed may have simply resulted from variation for these deleterious alleles or different degrees of inbreeding depression among lines.

The most basic assumption of trade-off theory predicts that, given limited resources, increased allocation of resources to one trait will lead to decreased availability of resources for any functionally associated trait (Roff, 2002). Under this assumption, negative genetic correlations between traits are predicted, where genotypes demonstrating high performance for one trait are predicted to have lower performance in the correlated trait (Stearns, 1989). This is based on the assumption, however, that different genotypes acquire comparable resources overall and vary primarily in the way in which they allocate those resources. It is also possible for there to be genetic variation for the ability to acquire resources, in which case some genotypes will have a much larger pool of resources to utilize. If there is substantially more genetic variation for resource acquisition than there is for resource allocation, positive rather than negative correlations between traits are predicted to occur (Van Noordwijk and De Jong, 1986), and this is what I observed in my data.

Positive correlations between traits are difficult to reconcile with high levels of genetic variation, because we would predict rapid fixation of alleles that confer improved general vigor. Houle (1991) extended the theoretical framework of Noordwijk and de Jong (1986) to

demonstrate that mutation selection balance is sufficient to maintain genetic variation for a pair of traits when variation in resource acquisition is high relative to variation in resource allocation. Deleterious recessive alleles contributing to the genetic variation I see among the inbred lines could therefore be maintained by mutation selection balance. Reznick et al. (2000) offered a different explanation, suggesting that increased overall acquisition may itself be costly, and may have negative consequences in different environmental conditions. It is possible that the lines that perform poorly in the experimental environment of this study may have an advantage in a nutrient-poor environment, for example. In future studies, it would be valuable to test the performance of these lines in differing environments. While my data are consistent with a theoretical framework of acquisition variation, I cannot discount the possibility that my results are inflated artificially by inbreeding.

I observed a positive correlation between traits in the inbred lines, but I observed no such phenomenon among the outbred genotypes, suggesting that the positive correlation may have been artificially created by inbreeding. Recessive deleterious mutations can accumulate and persist in populations in part because they are hidden from selection in heterozygotes. During the formation of inbred lines, however, high numbers of these mutations are artificially made homozygous, resulting in inbreeding depression. Inbreeding depression is predicted to cause spurious positive correlations between life history traits due to a depression in general vigor (Rose, 1984). A reduction in general vigor among the inbred lines is supported by examining the overall range of phenotypic values for inbred lines and outbred lines. For example, the range of average total progeny per female was 39.8-162.2 for inbred lines and 288.3-366.2 for outbred lines. This decrease in overall vigor may be due to reduced resource acquisition, and the theoretical predictions discussed in the previous paragraph would therefore still apply.

Regardless of whether this is the case or not, there is dubious applicability of the observations made in inbred flies to wild populations, especially since the experimentally outbred flies demonstrated no genetic correlations. As mentioned above, there is good evidence that genetic correlations between traits, whether positive or negative, are only observable in certain (often challenging) environmental conditions (Reznick et al., 2000). It is possible, then, that the outbred genotypes are unchallenged in the laboratory and would demonstrate a positive correlation if they were reared in a more stressful environment, but this requires additional investigation.

While antagonistic pleiotropy is unlikely in this experiment, it is possible for negative genetic correlations to be obscured by acquisition level variation or reduced general vigor (Houle, 1991; Fry, 1993). In the experiment using inbred lines, I detected a positive correlation between post-mating immune defense and egg production, where lines with high relative survival after mating also laid the most eggs. This correlation was much weaker (though still positive) when we compared post-mating immune defense and total adult progeny. From the egg production and progeny data, I calculated the proportion of eggs laid by each inbred line that successfully reached adulthood. I then found that this value of egg survivorship showed a non-significant weakly negative correlation with post-mating immune defense. Together, these data suggest that inbred lines with the highest immune defense after mating laid high numbers of eggs (relative to those with poorer post-mating defense), but that eggs from those high immune defense lines may have been less healthy and less likely to develop into viable adults.

The low level of eclosion success of progeny observed in inbred lines with high post-mating immune defense resulted in a mean number of progeny comparable to that of inbred lines with much poorer immune defense after mating. Evolutionary implications of this result are unclear, however, because even though lines with high post-mating immune defense may have

suffered a relative cost in terms of progeny success, the lines still performed generally better (high defense relative to virgins, moderate number of offspring) than "low post-mating immune defense" inbred lines (low defense relative to virgins, moderate number of offspring). While it is possible that I am detecting weak signals of antagonistic pleiotropy that may be more pronounced in a physiologically challenging situation, such as a nutrient-limited environment, I cannot determine that from these data.

In conclusion, these data do not fit a straightforward model of an evolutionary trade-off. Among inbred lines, variation for immune defense and reproduction appears to be mainly due to the presence of recessive deleterious mutations, which have the potential to cause positive correlations among traits. Outbred lines do not demonstrate the correlations seen in inbred lines, likely because inbreeding depression is alleviated in these lines. I cannot exclude the possibility of antagonistic pleiotropy however, as it could be obscured by resource acquisition variation or visible only under certain experimental conditions, but these data suggest it is more likely that genetic variation for the physiological trade-off between mating and immune defense does not result in a simple evolutionary tradeoff.

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Chapter 4.

Female *Drosophila melanogaster* suffer reduced defense against infection due to seminal fluid components

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Abstract:

Reduced defense against infection is commonly observed as a consequence of reproductive activity, but little is known about how post-mating immunosuppression occurs. In this work, we use *Drosophila melanogaster* as a model to test the role of seminal fluid components and egg production in suppressing post-mating immune defense. We also evaluate whether systemic immune system activity is altered during infection in mated females. We find that post-mating reduction in female defense depends critically on male transfer of sperm and seminal fluid proteins, including the accessory gland protein known as "sex peptide." However, the effect of these male factors is dependent on the presence of the female germline. We find that mated females have lower antimicrobial peptide gene expression than virgin females in response to systemic infection, and that this lower expression correlates with higher systemic bacterial loads. We conclude that, upon receipt of sperm and seminal fluid proteins, females experience a germline-dependent physiological shift that directly or indirectly reduces their overall ability to defend against infection, at least in part through alteration of humoral immune system activity.

1. Introduction:

Evidence that immune defense is involved in trade-offs with multiple life-history traits is abundantly apparent in a diversity of organisms ranging from invertebrates such as snails and insects to birds and mammals (reviewed in Schmid-Hempel, 2003; Sheldon and Verhulst, 1996). Defense against systemic infection by many bacterial pathogens (measured as resistance to infection or survival after infection) is reduced by mating in female *D. melanogaster* (Fedorka et al., 2007; Short and Lazzaro, 2010). In the present study, we investigate the immunological and reproductive bases for this post-mating depression in immune defense.

The insect immune system consists of multiple components, including the cellular immune response, the humoral immune response, and melanization. The cellular response functions mainly in the encapsulation or phagocytosis of parasites and pathogens, respectively (reviewed in Lemaitre and Hoffmann, 2007). The humoral immune response is activated upon detection of bacteria and fungi in the hemocoel. It includes production of antimicrobial peptides by the fat body and is stimulated when pattern recognition receptors recognize microbial cell wall compounds and trigger signaling through the Toll and IMD pathways (reviewed in Wang and Ligoxygakis, 2006). Melanization occurs in response to wounding, parasitization or infection and is regulated by the enzyme phenoloxidase (reviewed in Cerenius and Söderhäll, 2004).

These immune system components have been shown to be important for overall defense against infection in insects, which is defined as the ability to tolerate or eliminate infection (Ayres and Schneider, 2008). For this reason, quantitative immune system activity is often measured as a proxy for overall immune defense, under the implicit assumption that increased immune system activity correlates with heightened resistance to infection. This may or may not be the case (Fedorka et al., 2007), and this uncertainty can complicate the interpretation of immunity studies, an issue that has specifically been raised in the context of interactions between mating and immune defense (Lawniczak and Barnes et al., 2007). Regardless, much of the evidence for trade-offs between immune defense and reproductive success comes from studies demonstrating that mating and/or reproduction reduces proximal measures of systemic immune system activity or capability. In damselflies, the ability to encapsulate a foreign object inserted into the hemocoel decreases with increasing oviposition in females (Siva-Jothy et al., 1998), and sperm storage is negatively correlated with encapsulation ability in leaf-cutting ant queens (Baer and Armitage et al., 2006). In the beetle *Tenebrio molitor*, mating results in a decrease in

phenoloxidase activity in both males and females (Rolff and Siva-Jothy, 2002). Mating has mixed effects on the immune system of the cricket *Allonemobius socius,* reducing hemocyte number, encapsulation ability and lytic activity in both males and females, but increasing phenoloxidase activity in females (Fedorka et al., 2004).

While measurements of immune system activity certainly are informative, increases in immune activity do not always correlate with improved tolerance of infection or with heightened ability to eliminate pathogens (Adamo, 2004; Lawniczak and Barnes et al., 2007; Viney et al., 2005). For this reason, it is informative to also assess the efficacy of immune defense, which we measure in this study as the ability to fight and/or survive systemic infection. In *D. melanogaster*, multiple studies have investigated how mating affects both immune system activity and organism-level defense against infection. Females have been shown to demonstrate a short-term increase in the expression of at least one and often many antimicrobial peptide (AMP) genes after mating, at least in the reproductive tract and possibly in other tissues (Fedorka et al., 2007; Innocenti and Morrow, 2009; Kapelnikov et al., 2008; Lawniczak and Begun, 2004; Mack et al., 2006; McGraw et al., 2004; Peng et al., 2005b; Wigby et al., 2008). These data would seem to predict higher immunocompetence after mating. In fact, however, female *D. melanogaster* suffer reduced ability to defend against infection by pathogenic bacteria after mating (Fedorka et al., 2007; Short and Lazzaro, 2010), although the ability to eliminate non-pathogenic bacteria injected into the body cavity is not compromised (McKean and Nunney, 2005; Wigby et al., 2008). As of now, no mechanism has been demonstrated for the observed reductions in defense against infection after mating. Notably, all previous studies documenting the increase in AMP expression after mating have been performed using uninfected females.

Whether mating affects AMP expression in flies suffering from pathogenic infection remains an important but untested question.

During copulation, males transfer sperm and seminal fluid proteins in their ejaculates. Seminal fluid proteins, especially those made in the male accessory glands (accessory gland proteins, or Acps), have dramatic effects on female behavior and physiology. For example, Acp36DE causes conformational changes of the uterus (Avila and Wolfner, 2009) and is required for proper sperm storage after mating (Neubaum and Wolfner, 1999). Acp26Aa (ovulin) stimulates ovulation in mated females for approximately one day post-mating (Heifetz et al., 2000; Herndon and Wolfner, 1995). The Acp known as sex peptide (SP, also called Acp70A) has many effects on mated females, including reducing their receptivity to subsequent mating (Chapman et al., 2003; Chen et al., 1988; Liu and Kubli, 2003), promoting proper release of sperm from female storage organs (Avila et al., 2010), increasing intake of food (Carvalho et al., 2006) and decreasing siesta sleep (Isaac et al., 2010). SP has also been shown to be at least in part responsible for increased AMP gene expression in females after mating (Domanitskaya et al., 2007; Peng et al., 2005b). Interestingly, however, SP induces increases in juvenile hormone III-bisepoxide production in corpora allata incubated *in vitro* (Moshitzky et al., 1996), and juvenile hormone (JH) has been shown to suppress immune system activity (Flatt et al., 2008; Rolff and Siva-Jothy, 2002). Furthermore, seminal fluid, particularly SP, stimulates long-term increases in egg production (Chen et al., 1988; Soller et al., 1997), and egg production has been shown to trade-off physiologically (Fellowes et al., 1999) and evolutionarily (McKean et al., 2008) with immune defense. It is therefore possible that, despite inducing short-term modest increases in AMP expression, SP and other ejaculate components might cause overall reductions in systemic defense against infection. To begin to elucidate the mechanism by which females

suffer reduced defense against infection after mating, we tested the effect of mating on expression of immune genes during infection and used genetic manipulations to identify critical steps in copulation and reproduction that depress immune defense.

2. Methods:

2.1. Fly stocks and maintenance: Wild type flies are Canton S (CS) in all cases. "Spermless" males and "eggless" females are *tud*^{1} *bw sp/CS* and are generated from a cross between *tud*^{1} *bw sp* females and CS males. *tud*^{1} is a recessive maternal effect mutation, and offspring of *tud*^{1} mothers fail to form a germline. Sons of *tudor* females do transfer accessory gland proteins during mating. Egg-producing control females, which serve as a genotype control for eggless females, are also *tud*^{1} *bw sp/CS*. However, they are generated from a cross between *tud*^{1} *bw sp*/*CyO* females and CS males, and therefore produce eggs normally. "Spermless/Acpless" (DTA-E) males have ablated accessory glands due to expression of diphtheria toxin subunit A in their accessory gland main cells (Kalb et al., 1993). They fail to produce sperm and main cell accessory gland proteins (Kalb et al., 1993). Sex peptide null males are SP^{0}/Δ^{130} and were generated from a cross between SP^{0}/*TM3, Sb ry* and Δ^{130}/*TM3, Sb ser* (Liu and Kubli, 2003). Sex peptide null flies were donated by Eric Kubli.

All flies were reared on standard Cornell media (8.3% glucose, 8.3% Brewer's yeast, and 1% agar, plus 0.04% phosphoric acid and 0.4% propionic acid added to inhibit microbial growth in the food). Flies were kept at 24°C on a 12 hour light-dark cycle.

2.2. Mating setup: Male and female virgins were collected within 8 hours of eclosion, separated by sex, and aged in groups of ~25 with *ad libitum* access to food. All flies were three days post-eclosion at the time of mating. The day before each experiment, females were

anaesthetized on CO_2, put into individual glass mating vials, randomly allocated to a mating treatment and allowed to recover overnight. Females that were to remain virgins were anaesthetized and also put into individual vials. The following day, single, unanaesthetized males were aspirated into vials containing females within three hours of incubator "dawn." Mating pairs that copulated for less than 15 minutes were discarded before infection in order increase our confidence that the male had adequate time to transfer the full complement and amount of ejaculate (where appropriate) and to verify that females mated to mutant males mated for similar lengths of time as females mated to wild type males. More than 95% of all copulations lasted for longer than 15 minutes, so the number discarded from our experiment represents a small fraction of the total number of copulating pairs. After mating, males were removed, and females that ceased mating within roughly 10 minutes of each other were combined into vials of ~10 flies per vial. Virgin females were combined in similarly sized groups to control for possible housing effects.

2.3. Bacterial infection: Mated females were infected 2-3 hours after mating unless otherwise noted. In all cases, control virgin females were infected in parallel with their mated counterparts. Females were anaesthetized on CO_2 and pierced in the thorax with a 0.15mm anodized steel needle (FST) dipped in a dilute overnight culture of *Providencia rettgeri.* The strain of *P. regtteri* used in this experiment is a natural bacterial pathogen of *D. melanogaster,* and resistance to it has been shown to be reduced by mating (Short and Lazzaro, 2010). *P. rettgeri* is a moderate bacterial pathogen, causing ~40% mortality over 3-7 days in virgin *D. melanogaster* infected under our procedures. Overnight cultures were started from a single bacterial colony, grown overnight at 37ºC to saturation in liquid Luria Broth (LB), then diluted in additional LB to A_{600}=1.0 (±0.05).

2.4. Bacterial load assay: Bacterial load was assayed 24 hours after infection in all cases with the exception of the data presented in Figure 4.2, when we assayed bacterial load at multiple time points after infection. To determine bacterial load, females were pooled in groups of 3 and homogenized in 500µl LB with a sterile pestle. Homogenates were diluted as described below with additional LB, and 50µl of the homogenate was plated onto LB agar using a WASP 2 spiral plater (Microbiology International, Bethesda, MD, USA). For measurements taken 24 hours after infection, the homogenate was diluted 1:1000 prior to plating. For measurements taken 12 hours after infection, the homogenate was diluted 1:100 prior to plating. Plates were grown overnight at 37ºC, and resulting colonies were counted using a ProtoCOL plate counting system (Microbiology International) to estimate the number of colony forming units in each pool of three flies at the time of homogenization. Plates were routinely checked for contamination by visual inspection of colony morphology. Additionally, we periodically amplified 16S rDNA from a subset of colonies using the primers fd1 and rp2 (Weisburg et al., 1991), and amplified the same sequence in colonies grown from a pure freezer stock of *P. rettgeri.* We performed a restriction digestion on the amplifications from both the experimental colonies and the pure stock using *MspI*, ran each digest product on a 1% agarose gel, and compared banding patterns. In all cases, the banding pattern of the experimental colonies was an exact match to that of the positive control colonies from the freezer stock. Control sets of females were wounded with a sterile needle, and the plates from these flies yielded zero colonies.

2.5. Survival assay: Immediately after infection, females were placed into vials in groups of 10 with *ad libitum* access to food. Females were observed shortly after this to confirm that they had recovered from infection, and those that did not recover were not included in the experiment. Survival was recorded daily for five days, with surviving females from all

treatments transferred to new media approximately every other day. Subsets of females from each treatment were pierced with a sterile needle to verify that survival differences between treatments were a consequence of infection and not injury. In all cases, females pierced with a sterile needle demonstrated negligible mortality (0% for most treatments and < 5% for all treatments)

2.6. Measurement of immune system activity: At 0, 4, 12 and 24 hours after infection, mated CS females and control virgins were sorted into pools of 10, snap frozen in TRIZOL reagent (Invitrogen) and stored at -80ºC. Total RNA was isolated using the TRIZOL manufacturer's recommended protocol, dissolved in RNase-free water, and stored at -80ºC. We then treated approximately 500ng of total nucleic acid from each sample with DNase (Promega) in order to eliminate any residual DNA contamination and manufactured cDNA using M-MLV reverse transcriptase (Promega). Quantitative real-time PCR was performed using the ABI Prism 7000 Sequence Detection System (Applied Biosystems). Expression levels of all AMP genes are reported relative to expression of *RpL32* (also known as *rp49*), and results were verified using *Actin 5C* as an additional reference control gene. To quantify expression of *Attacin A, Attacin B, Metchnikowin, RpL32 and Actin 5C*, we used Power SYBR green PCR master mix (Applied Biosystems). To quantify expression of *Defensin, Drosomycin and Diptericin A,* we used gene-specific Taqman fluorescent probes (Applied Biosystems). Primers and primer/probe sequences are available upon request.

2.7. Statistical analysis: In all experiments where bacterial load was measured, the data were natural log transformed. We then fit a mixed model ANOVA using SAS (SAS Institute) to determine the effect of mating treatment and, where appropriate, time after mating (Figure 4.1), female genotype (Figure 4.5A), and the interactions between mating treatment and these

additional factors. The residual errors of the ANOVA were adequately normally distributed. To assess the effect of mating at different time points, we sorted by time point and performed contrasts between mating statuses within each subset of data. Replicate experiment was included in each ANOVA as a random effect. In cases with more than two mating statuses (Figures 4.3A, 4.4A and 4.5A), we performed a Tukey's test to conduct pairwise comparisons between treatments and to correct for multiple comparisons.

In all experiments measuring survival, we assessed the effect of mating status using Cox regression analysis in SAS (SAS Institute). Event data (where an "event" = death) were recorded for flies from each mating status, and flies that were still alive at the end of the observation period were treated as censored data. Mating treatment and replicate were included as factors in all regression analyses, and in experiments with more than two mating treatments, comparisons between mating treatments of interest were performed using contrast statements within the regression analysis. A Bonferroni correction was applied in these situations to correct for multiple testing.

In the analysis of gene expression data, technical replicates for all measurements were averaged and the following model was fitted to the average critical threshold values for all measured AMP genes: $Y_{ijkl} = \mu + \text{RpL32 expression} + \text{time}_i + \text{mating status}_j + \text{gene}_k + \text{experimental replicate}_l + \text{mating status}_j*\text{gene}_k + \text{mating status}_j*\text{time}_i + \text{gene}_k*\text{time}_i + \text{mating status}_j*\text{gene}_k*\text{time}_i$, where time ($i$ = 1,4), mating status (j = 1,2) and gene (k = 1,6) are fixed effects and experimental replicate (l = 1,2) is random. Because a significant mating status*time interaction was observed, the data were then sorted by time post-infection and the following model was applied to data from each time point: $Y_{ijk} = \mu + \text{RpL32 expression} + \text{mating status}_i + \text{gene}_j + \text{experimental replicate}_k + \text{mating status}_i*\text{gene}_j$, where mating status ($i$ = 1,2) and gene (j

= 1,6) are fixed effects and experimental replicate (k = 1,2) is random. Least squares means for the mating status*gene interaction were obtained from these analyses and, for each time point, we subtracted the mated LS means from the virgin LS means for each gene. We then plotted this difference along a zero axis, where a bar above the zero axis represents a higher level of gene expression at that time point in mated females relative to virgin controls and a bar below the zero axis represents a lower level of expression in mated females relative to virgins. These differences and the standard errors of the differences were determined using the lsmestimate command in SAS (SAS Institute).

3. Results:

3.1. The effect of mating on female immune defense lasts for at least twenty-four hours after mating.

Mated *D. melanogaster* females suffer a reduction in immune defense against bacterial infection that begins as early as 2.5 hours after mating and may persist for several additional hours beyond this time point (Fedorka et al., 2007; Short and Lazzaro, 2010). To test for persistence of mating-induced immunodepression in our experimental context, we assayed bacterial load in *D. melanogaster* females (wild type strain Canton S, or CS) infected at 2.5, 6, 12 and 24 hours after mating cessation. We also assayed for differences in survivorship of infection in females infected at 2.5 and 26.5 hours after mating cessation. In both the bacterial load and survival experiments, virgin and mated females were infected in parallel with the bacterium *Providencia rettgeri* by septic pinprick to the thorax. Systemic bacterial load was recorded 24 hours after infection, and mortality was scored immediately following infection and every day thereafter for a total of five days. For each of the four post-mating infection time

points, we found that mated females had significantly higher bacterial loads than virgin control females (Figure 4.1A; 2.5 hours post-mating, $p = 0.0002$; 6 hours post-mating, $p = 0.0058$; 12 hours post-mating, $p = 0.0009$; 24 hours post-mating, $p = 0.0116$). We also found that mated females had significantly lower survival than virgin controls over time when infected at both 2.5 (Figure 4.1B, $p < 0.0001$) and 26.5 hours after mating (Figure 4.1C, $p = 0.0028$). Thus, the effect of mating on defense against systemic bacterial infection is persistent with no sign of decline for at least 24 hours post-mating.

3.2. Mated females demonstrate higher bacterial loads but lower AMP expression early in bacterial infection.

Production of antimicrobial peptides (AMPs) is one important component of the immune response in insects (reviewed in Lemaitre and Hoffmann, 2007). Multiple studies have reported short-term increases in expression of at least one and often multiple AMP genes after mating, where expression level changes have been measured either in the female reproductive tract (Mack et al., 2006; Kapelnikov et al., 2008) or in undissected whole flies (Fedorka et al., 2007; Innocenti and Morrow, 2009; Lawniczak and Begun, 2004; McGraw et al., 2004; Peng et al., 2005b; Wigby et al., 2008). Only two of these studies (Fedorka et al., 2007; Wigby et al., 2008), however, measured organism-level defense against infection in parallel with changes in AMP expression. Fedorka et al. (2007) reported a significant reduction in defense against pathogenic infection after mating, and Wigby et al. (2008) saw no effect of mating on the female's ability to clear non-pathogenic *E. coli* from the hemocoel. In neither of these studies does a mating-induced increase in AMP gene expression result in increased immune defense against systemic infection. We posited that, over the course of an infection, mating might actually compromise

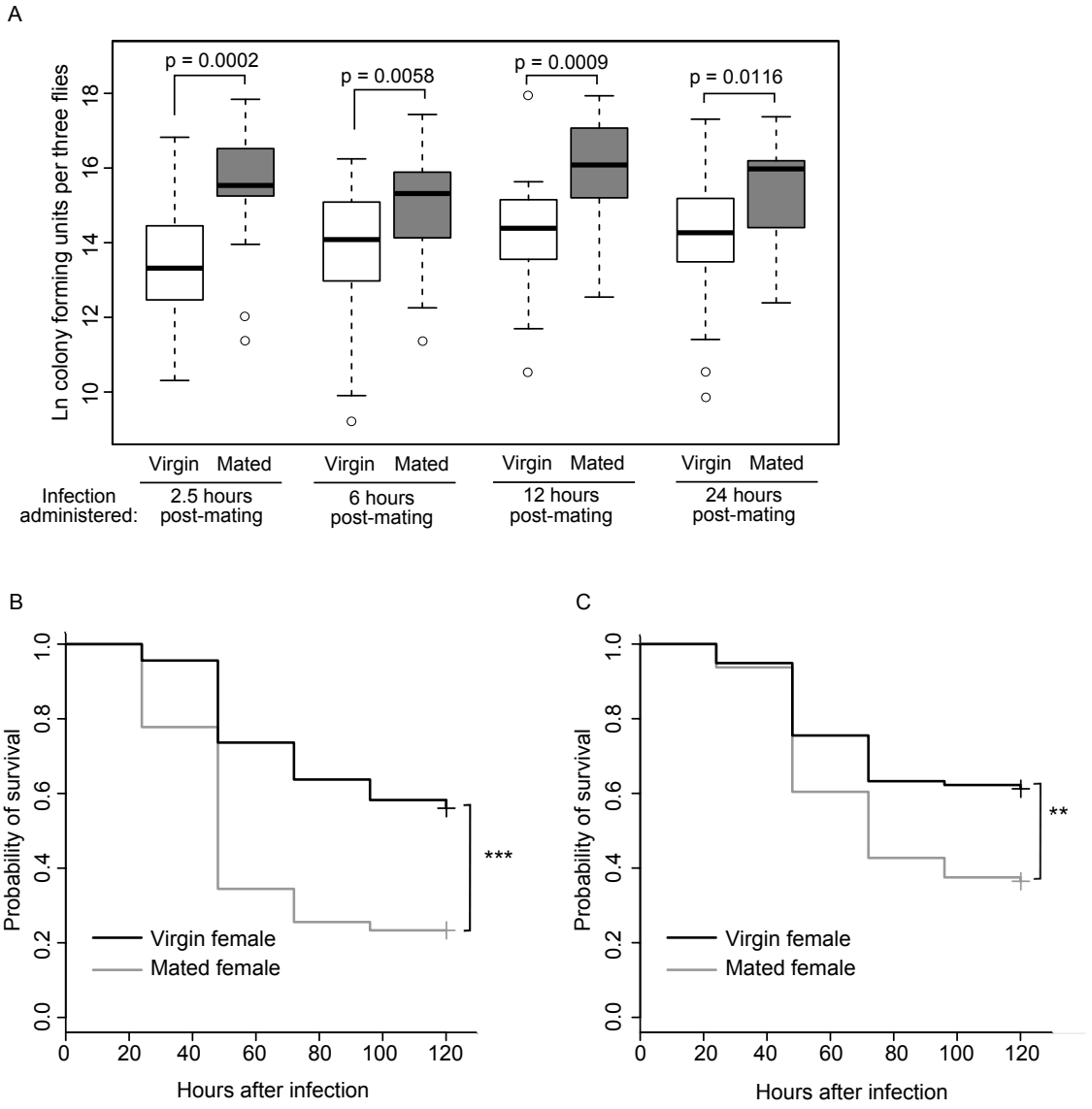

Figure 4.1. The effect of mating on immune defense lasts for at least 24 hours after mating. (A) Bacterial load levels of mated Canton S females were higher than those of virgin controls when infected at 2.5 hours ($p = 0.0002$), 6 hours ($p = 0.0058$), 12 hours ($p = 0.0009$) and 24 hours ($p = 0.0116$) after mated flies finished copulating. All flies were infected with *P. rettgeri* and virgin controls were aged, housed and infected in parallel with mated females for each time point. Each data point consists of a pool of three females, and the number of pools for each treatment are as follows: for 2.5 hours, $n_{mated} = 22$ and $n_{virgin} = 22$, for 6 hours, $n_{mated} = 24$ and $n_{virgin} = 26$, for 12 hours, $n_{mated} = 21$ and $n_{virgin} = 20$, and for 24 hours, $n_{mated} = 21$ and $n_{virgin} = 22$. Data were collected over three replicate experiments. Flies given a sterile wound always yielded zero colonies (data not shown). **(B,C)** Survival over time of mated Canton S females was significantly lower than that of virgin controls when infected at 2.5 hours (B, $p < 0.0001$) and 26.5 hours (C, $p = 0.0028$) after mated females finished copulating. All flies were infected with *P. rettgeri* and kept in groups of ~10 flies. Sample sizes are as follows: for 2.5 hours, $n_{mated} = 90$ and $n_{virgin} = 91$, for 26.5 hours, $n_{mated} = 96$ and $n_{virgin} = 98$. Mortality was recorded each day for five days after infection, and data were collected over two replicate experiments. Survival curves were estimated using the Kaplan-Meier method. Control flies given a sterile wound had negligible mortality over the course of the experiment (less than 1%) regardless of mating treatment (data not shown). ** $p < 0.01$, *** $p < 0.001$.

systemic immune system activity relative to virgin females, thus explaining the observed reduction in overall defense.

To test post-infection immune performance in mated females relative to virgins, we measured the transcript levels of several AMP genes at different time points after infection in both mated females and virgin controls. Note that for this experiment, and for all subsequent experiments, females were infected at 2.5 hours post mating. Levels of AMP gene expression increased dramatically over the course of infection, but the level of immune system induction varied significantly between mated and virgin females in a time-specific manner (Table 4.1), with mated females showing lower AMP gene expression at 4 and 12 hours post-infection (Figure 4.2, 4 hours $p = 0.001$, 12 hours $p < 0.0001$) but higher gene expression after 24 hours (Figure 4.2, $p < 0.0001$). This pattern was consistent across six AMP genes measured (Table 4.1, Figure 4.2). Interestingly, we did not observe the induction of AMP gene expression reported by others in response to mating itself (Figure 4.2, $p = 0.1019$) (Lawniczak and Begun, 2004; McGraw et al., 2004). Systemic bacterial load did not differ significantly between mated and virgin females at four hours after infection (Figure 4.2, $p = 0.9540$), but mated females have significantly higher systemic bacterial loads at 12 hours post-infection (Figure 4.2, $p = 0.0003$) and 24 hours post-infection (Figure 4.1, $p = 0.0002$). We infer that the lower early AMP gene expression in mated females relative to virgins may contribute to the increased pathogen proliferation observed in mated females, and that the expression of immune system genes in mated females becomes higher than that in virgins at 24 hours post-infection due to greater sustained stimulation of the immune system by the correspondingly more severe infection (Figure 4.2).

Table 4.1: The effect of mating status on transcription levels of six AMP genes at four time points post-infection.

Factor	Effect type	d.f.	F value	P-value
RpL32 expression (CT value)				< 0.0001
Time	Fixed	3	3747.3	< 0.0001
Mating status	Fixed	1	3.64	0.0573
Gene	Fixed	5	1055.4	< 0.0001
Experimental rep	Random			0.4995
Mating status * gene	Fixed	5	1.41	0.2219
Mating status * time	Fixed	3	15.13	< 0.0001
Gene * time	Fixed	15	99.32	< 0.0001
Mating status * gene * time	Fixed	15	0.80	0.6756

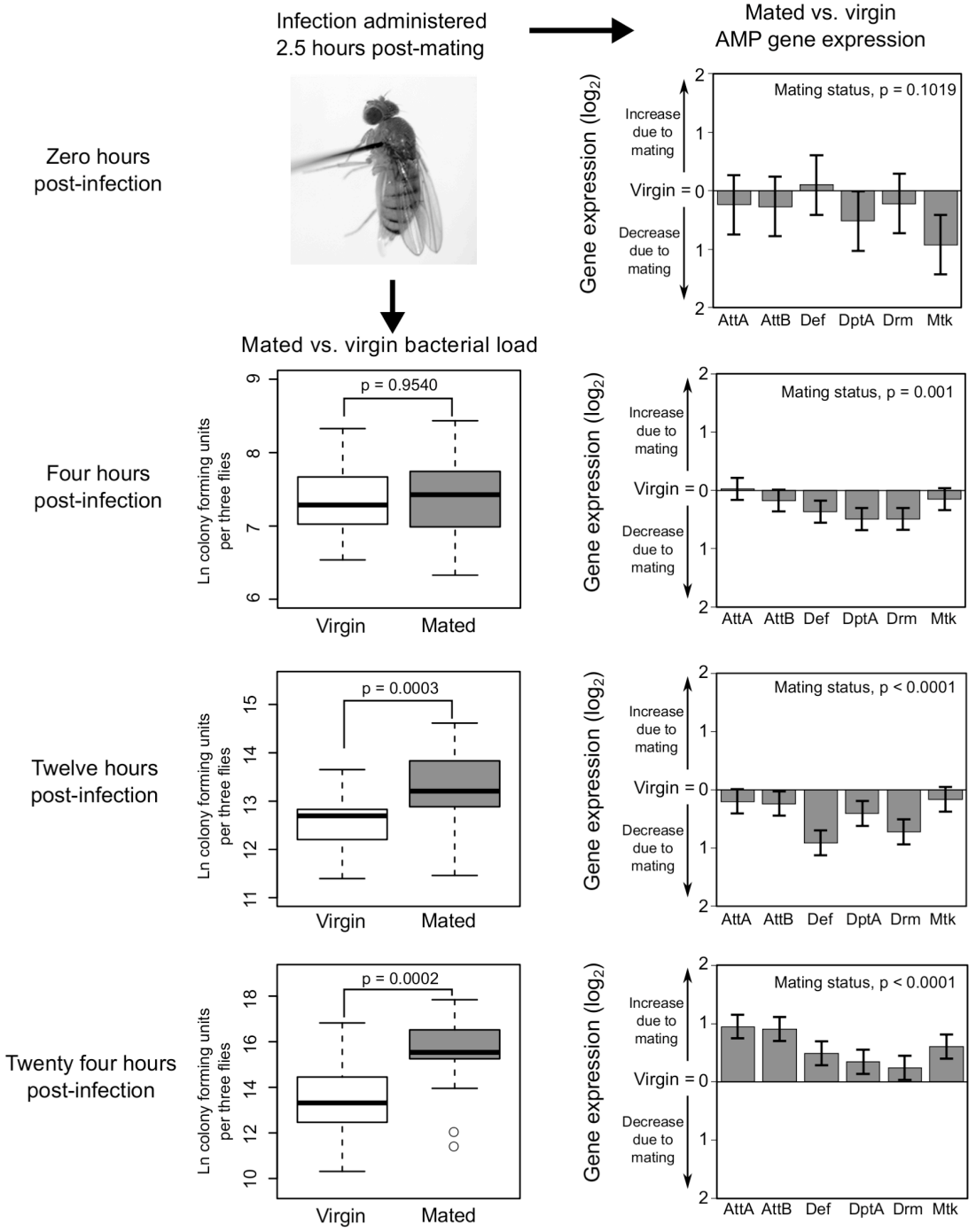

Figure 4.2. Antimicrobial peptide gene expression in mated females relative to virgins during the course of infection. Mated and virgin Canton S females were infected 2.5 hours after mating with a 1.0 A_{600} culture of *P. rettgeri.* Bacterial load was measured at 4, 12 and 24 hours after infection in independent experiments. Bacterial loads in mated females did not significantly differ from virgin females at 4 hours post infection (p = 0.9540) but did at 12 hours (p = 0.0003) and 24 hours post infection (p = 0.0002, data taken from Figure 1A). Each data point is a pool of three females, and the number of data points per treatment are as follows: at 4 hours, n_{mated} = 23 and n_{virgin} = 24, at 12 hours, n_{mated} = 23 and n_{virgin}

= 27, and at 24 hours, n_{mated} = 22 and n_{virgin} = 22. Flies given a sterile wound always yielded zero colonies (data not shown). AMP gene expression was assayed in a subsequent experiment at 0, 4, 12 and 24 hours after infection. Gene expression increased significantly over the course of infection in both virgin and mated females, but because the effect of mating status varied by time point, data were sorted by time and least squares means for each mating status/AMP gene combination were found by a mixed-model ANOVA. P-values reported on gene expression graphs are from these models and indicate the effect of mating status on overall AMP gene expression. Data are presented as the Log_2 fold difference in mated female LS means relative to virgin control LS means for each gene, where a bar above or below the virgin level of expression represents a higher or lower level of expression due to mating, respectively. Because the differences are Log_2, an increase of "1" corresponds to 2x the virgin level of gene expression at that time point, and a decrease of "1" corresponds to half the virgin level. Sample sizes are as follows: 0 hours, n_{mated} = 6, n_{virgin} = 6, 4 hours, n_{mated} = 8, n_{virgin} = 8, 12 hours, n_{mated} = 8, n_{virgin} = 8, 24 hours, n_{mated} = 8, n_{virgin} = 8, where each sample consists of a pool of 9-10 females collected over two replicate experiments.

3.3 Post-mating suppression in female immune defense depends on transfer of both sperm and sex peptide.

3.3.1. The role of sperm and Acps in female immune defense: Male-derived seminal fluid has many dramatic effects on female physiology (reviewed in Avila et al., 2011), so we hypothesized that seminal fluid signals might elicit changes in female immune defense. In order to determine whether sperm or accessory gland proteins elicit post-mating reductions in female defense, we compared bacterial load and survival after *P. rettgeri* infection in CS females from four different mating treatments: (1) virgin females and females mated to (2) wild type males, (3) males who do not produce sperm, or (4) males who produce neither sperm nor accessory gland proteins (Acps). Spermless males have the genotype tud^{1} *bw sp/CS* and are sons of *tudor* homozygous mutant mothers and CS fathers. Because *tudor* is a maternal effect mutation, these males lack pole cells and fail to form a germline (Boswell and Mahowald, 1985). Spermless/Acpless males have accessory glands whose main cells have been ablated by cell-specific expression of diptheria toxin subunit A (Kalb et al., 1993). These males also fail to produce sperm. By comparing the response of females mated to wild type males and females mated to spermless males, we can determine the specific importance of sperm. By comparing the response of females mated to spermless males to those mated to spermless/Acpless males, we can determine the additional effect of accessory gland proteins (Kalb et al., 1993).

Females that were mated to wild type males sustained significantly higher bacterial loads than did virgin females by 24 hours post-infection ($p < 0.0001$), but females mated to spermless/Acpless males sustained systemic bacterial loads equivalent to those of virgin females (Figure 4.3A, $p = 0.442$). Females mated to spermless males had bacterial loads that were intermediate between those of females mated to wild type males and those of virgin females

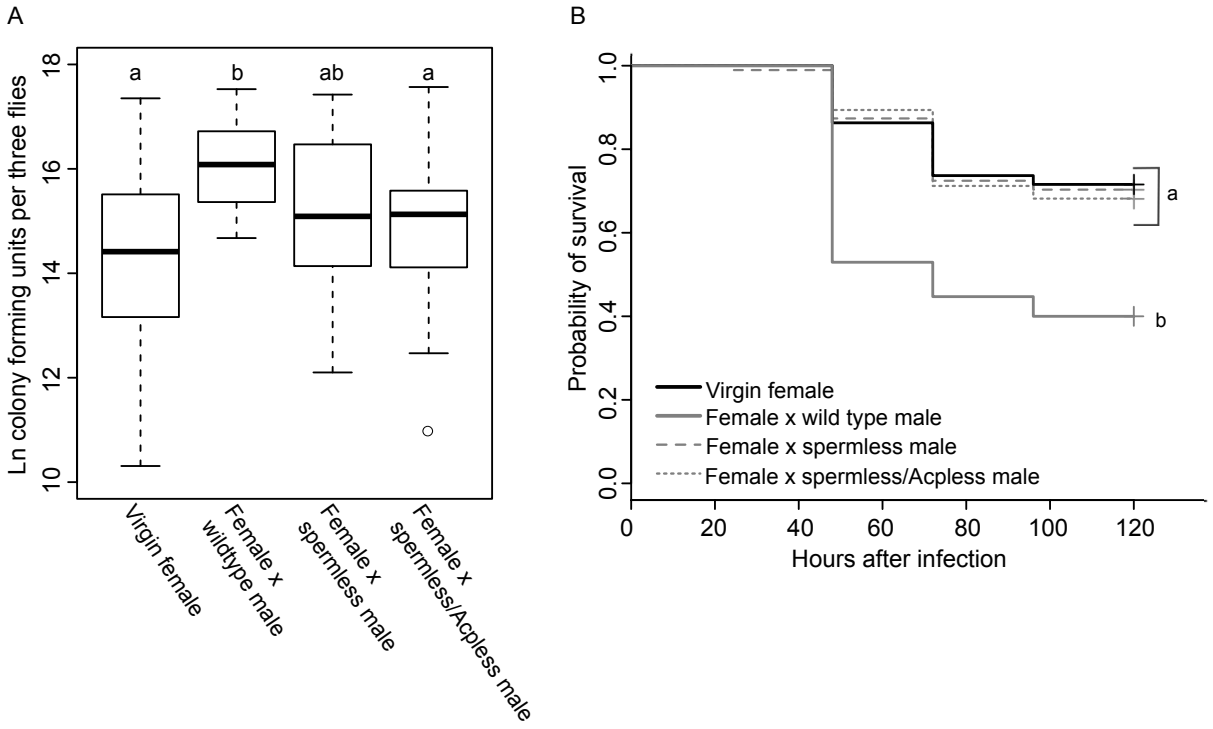

Figure 4.3. The effect of sperm and accessory gland protein transfer on post-mating female immune defense. Canton S females were assigned to one of four mating treatments: virgin (V), mated to CS males (M_{CS}), mated to spermless males (M_{SL}) or mated to spermless/Acpless males ($M_{SL/AcpL}$). In both experiments, females from all mating treatments were infected with a 1.0 A_{600} culture of *P. rettgeri.* (A) Bacterial load was assayed in females from all mating treatments and Tukey's test was used to make the following treatment comparisons: V vs. M_{CS}, $p < 0.0001$; V vs. M_{SL}, $p = 0.07$; V vs. $M_{SL/AcpL}$, $p = 0.442$; M_{CS} vs. M_{SL}, $p = 0.08$; M_{CS} vs. $M_{SL/AcpL}$, $p = 0.0084$; M_{SL} vs. $M_{SL/AcpL}$, $p = 0.885$. Each data point is a pool of three flies, and the number of data points collected for each treatment are as follows: $n_{virgin} = 26$, $n_{\times CSmale} = 29$, $n_{\times spermless} = 26$, $n_{\times spermless/Acpless}=25$. Samples were collected over three replicate experiments, and flies given a sterile wound always yielded zero colonies (data not shown). (B) Survival was assayed for females from all mating treatments, and Cox regression analysis was used to determine the effect of mating treatment on survival. Independent contrasts were performed within the regression analysis, and the Bonferroni corrected p-value for the six pairwise comparisons is 0.0083. V vs. M_{CS}, $p < 0.0001$; V vs. M_{SL}, $p = 0.8705$; V vs. $M_{SL/AcpL}$, $p = 0.727$; M_{CS} vs. M_{SL}, $p = 0.0001$; M_{CS} vs. $M_{SL/AcpL}$, $p = 0.0011$; M_{SL} vs. $M_{SL/AcpL}$, $p = 0.8417$. Sample sizes: $n_{virgin} = 95$, $n_{\times CSmale} = 85$, $n_{\times spermless} = 95$, $n_{\times spermless/Acpless}=66$. Each data point represents a single fly and samples were collected over two replicate experiments. Flies given a sterile wound had 0% mortality in all treatments.

(Figure 4.3A; virgin vs. female × spermless male, p = 0.0781, female × wild type male vs. female × spermless male, p = 0.0831).

Females mated to spermless/Acpless males also survived their infections significantly better than females mated to wild type males (p = 0.0011), and were equivalent to virgin females (Figure 4.3B, p = 0.7270). Interestingly, females mated to males lacking sperm were significantly more likely to survive infection than females mated to wild type males (p = 0.0001), also surviving equivalently to virgin females (Figure 4.3B, p = 0.8705). Thus, failure to transfer sperm alone was sufficient to eliminate the effect of mating on survival. The probability of survival for females mated to spermless/Acpless males was not significantly different from that of females mated to spermless males (Figure 4.3B, p = 0.8417).

3.3.2. The role of sex peptide in female immune defense: Previous studies have shown an important role for the Acp known as sex peptide (SP) on female physiology and behavior (*e.g.* Avila et al., 2010; Carvalho et al., 2006; Chen et al., 1988; Isaac et al., 2010; Liu and Kubli, 2003; Moshitzky et al., 1996; Soller et al., 1997). The effects of SP persist for days in the female, but only if sperm are also successfully transferred and stored (Manning, 1962). SP is tethered to sperm tails in the female reproductive tract, and it is thought that this allows SP to persist in the female and to be slowly released over time (Peng et al., 2005a). We hypothesized that SP may indeed play a role in female immune defense, but that its effect may be dependent on sperm transfer. We therefore contrasted systemic bacterial load and survival after infection of females from four different mating treatments: (1) virgin females and females mated to (2) wild type males, (3) spermless males or (4) males null for sex peptide (Liu and Kubli, 2003). SP null males ($SP^0/\Delta 130$) carry a null mutation of the sex peptide gene uncovered by deficiency Δ130 and fail

to produce functional sex peptide, but are normal in their other seminal fluid components, sperm production, and mating biology.

As in our previous experiments, females mated to wild type males had significantly higher bacterial loads than virgin females at 24 hours post-mating (Figure 4.4A, $p < 0.0001$). Again, females that failed to receive sperm during copulation demonstrated intermediate bacterial loads, significantly lower than those of females mated to wild type males ($p = 0.0225$) but still significantly higher than those of virgin females (Figure 4.4A, $p = 0.0027$). Females mated to sex peptide null males showed this same pattern, exhibiting bacterial loads significantly lower than those of females mated to wild type males ($p = 0.0136$) but significantly higher than virgins (Figure 4.4A, $p = 0.0097$). Bacterial loads of females mated to spermless males or to SP null males were equivalent (Figure 4.4A, $p = 0.9933$). These data are consistent with the hypothesis that proper delivery of sex peptide is crucial for immune defense to be reduced after mating, but also indicate that other components of the seminal fluid must contribute to mating-induced changes in bacterial load since elimination of sex peptide is not sufficient to return females to virgin defense levels.

Failure to transfer sex peptide is sufficient to entirely eliminate the effect of mating on female survival of infection. Females mated to SP null males were significantly more likely to survive infection than females mated to wild type males ($p < 0.0001$), and their survival did not significantly differ from that of virgin females (Figure 4.4B, $p = 0.8681$). Females mated to spermless males showed this same pattern, and the survivorships of females mated to either spermless or SP null males were equivalent (Figure 4.4B, $p = 0.1311$)

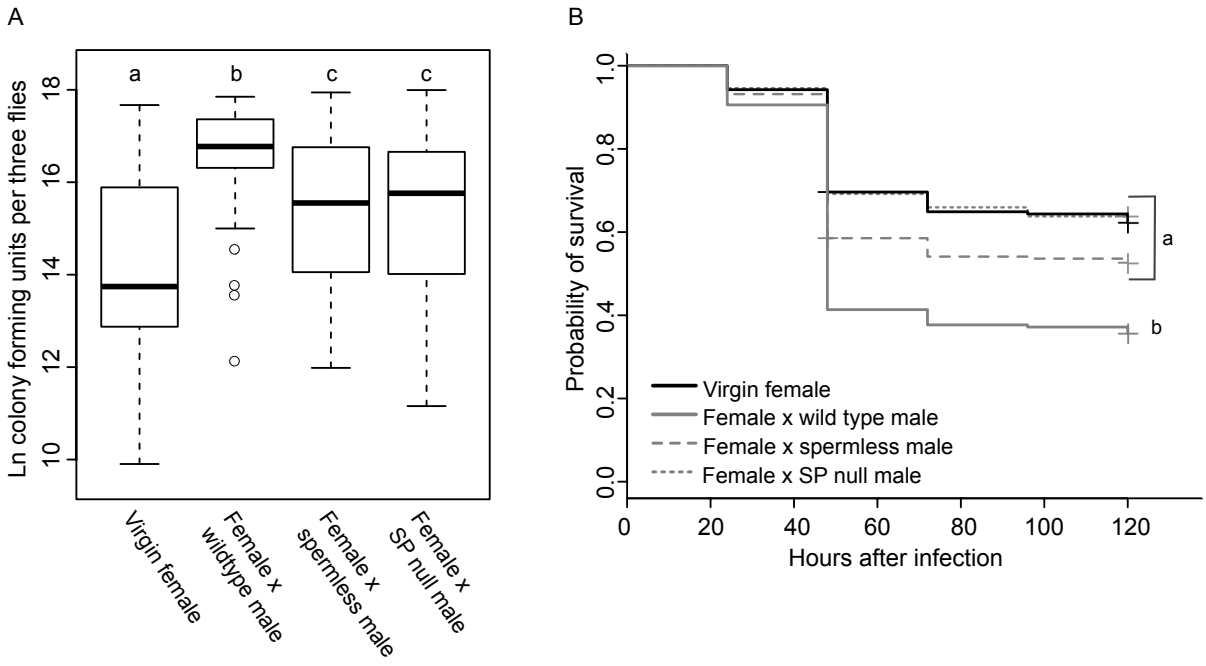

Figure 4.4. The effect of sperm and sex peptide transfer on post-mating female immune defense. CS females were assigned to one of four mating treatments: virgin (V), mated to CS males (M_{CS}), mated to spermless males (M_{SL}) or mated to sex peptide null males (M_{SP}). In both experiments, females from all mating treatments were infected with *P. rettgeri.* (A) Bacterial load was assayed in females from all mating treatments 24 hours after infection and Tukey's test was used to make the following comparisons: V vs. M_{CS}, $p < 0.0001$; V vs. M_{SL}, $p = 0.0027$; V vs. M_{SP}, $p = 0.0097$; M_{CS} vs. M_{SL}, $p = 0.0225$; M_{CS} vs. M_{SP}, $p = 0.0136$; M_{SL} vs. M_{SP} males, $p = 0.993$. Each data point is a pool of three flies, and the number of data points for each treatment are as follows: $n_{virgin} = 45$, $n_{\times CSmale} = 40$, $n_{\times spermless} = 42$, $n_{\times SPnullmale}=37$. Samples were collected over five replicate experiments, and flies given a sterile wound always yielded zero colonies (data not shown). (B) Survival was assayed for all mating treatments, and Cox regression analysis was used to determine the effect of mating treatment on survival. Independent contrasts were performed within the regression analysis, and the Bonferroni corrected p-value for the six pairwise comparisons is 0.0083. V vs. M_{CS}, $p < 0.0001$; V vs. M_{SL}, $p = 0.0844$, V vs. M_{SP}, $p = 0.86$; M_{CS} vs. M_{SL}, $p = 0.007$; M_{CS} vs. M_{SP}, $p < 0.0001$; M_{SL} vs. M_{SP}, $p = 0.13$. Sample sizes: $n_{virgin} = 191$, $n_{xCSmale} = 191$, $n_{xspermless} = 205$, $n_{xSPnullmale}=175$. Each data point represents a single fly and samples were collected over five replicate experiments. Flies given a sterile wound had 0% mortality.

3.4. Females that fail to produce eggs demonstrate no effect of mating on immune defense

We hypothesized that after mating, females may experience a shift in physiological and molecular signaling toward a state that maximizes vitellogenesis and egg production, and that any such shift might occur at the cost of immunocompetence. If this hypothesis is correct, we would predict that mated females that lack a germline and therefore fail to produce eggs would not demonstrate an immunological cost of mating. To test this hypothesis, we generated genetically identical females that did or did not produce eggs. "Eggless" females (*tud*^{1} *bw sp/CS*) were produced from a cross between *tudor* mutant females (*tud*^{1} *bw sp*) and CS males. The maternal effect of *tudor* results in daughters that fail to develop a germline and therefore cannot produce eggs. "Egg producing" control females of the genotype *tud*^{1} *bw sp/CS* were generated from a cross between *tud*^{1} *bw sp/CyO* mothers and CS fathers. We then measured bacterial load and survival in mated and virgin egg-producing and eggless females. We found that egg producing females sustained significantly higher *P. rettgeri* loads due to mating (Figure 4.5A, $p = 0.0003$), but that the bacterial loads of mated eggless females were equivalent to those of virgin eggless females (Figure 4.5A, $p = 0.7792$). Similarly, mating resulted in significantly decreased survival of infection in egg producing females ($p = 0.0022$), but not in eggless females ($p = 0.7718$; Figure 4.5B). These results demonstrate the requirement of a female germ line in order for female immune defense to be affected by male seminal signals.

4. Discussion:

While evidence of evolutionary and physiological trade-offs between immune defense and life history traits is abundant, comparatively little is known about how trade-offs occur on a physiological or genetic level. In this work, we demonstrate that post-mating reductions in

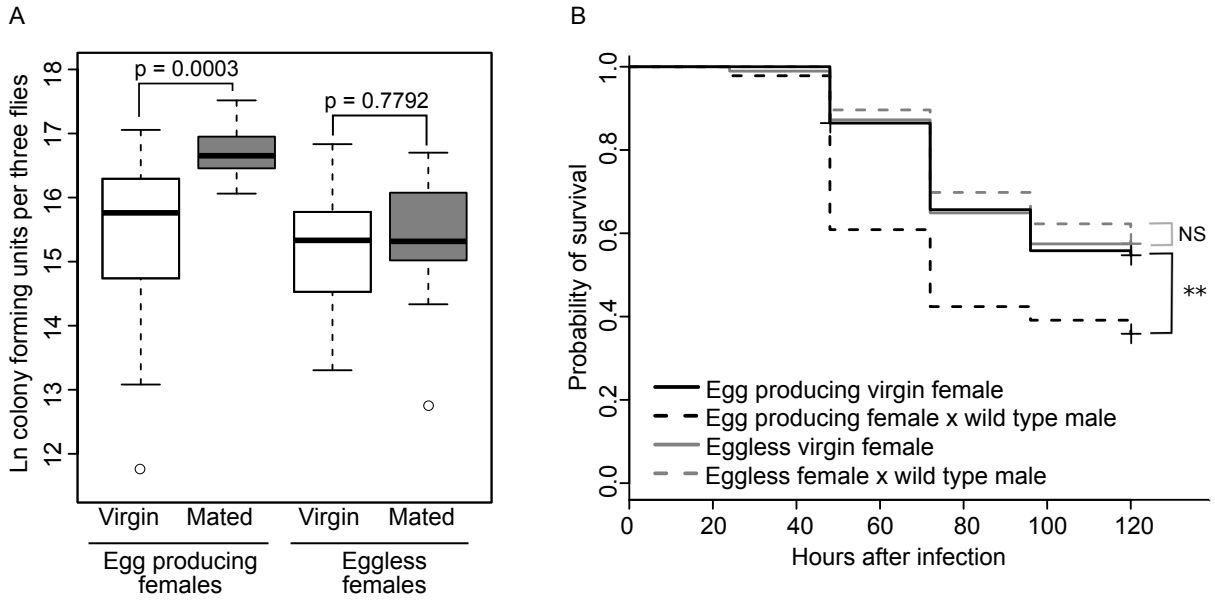

Figure 4.5. Females that fail to produce eggs do not demonstrate decreased immune defense after mating. Eggless females are *tudor bw sp/CS* (generated by *tudor bw sp/tudor bw sp* females x CS males) and have no germline. Egg producing females are *tudor bw sp/CS* (generated by *tudor bw sp/CyO* females x CS males) and have wild type egg production. All females were infected with *P. rettgeri* 2-3 hours after mated females completed copulation. (A) Egg producing females demonstrated a significant effect of mating on bacterial load ($p = 0.0003$), while eggless females did not ($p = 0.7792$). We assayed bacterial load in females 24 hours after infection. Sample sizes: $n_{virgin,\ egg\ producing} = 15$, $n_{mated,\ egg\ producing} = 16$, $n_{virgin,\ eggless} = 14$, $n_{mated,\ eggless} = 15$. Each data point consists of three pooled females and samples were collected over two replicate experiments, and flies given a sterile wound always yielded zero colonies (data not shown). (B) Egg producing mated females demonstrated significantly lower survival after infection compared to egg producing virgin females ($p = 0.0022$, Bonferroni corrected cutoff=0.025). Survival of eggless mated females was not significantly different from that of eggless virgin females ($p = 0.7718$). Sample sizes: $n_{virgin,\ egg\ producing} = 96$, $n_{mated,\ egg\ producing} = 92$, $n_{virgin,\ eggless} = 94$, $n_{mated,\ eggless} = 106$. Each data point represents a single fly and samples were collected over two replicate experiments. Females given a sterile wound had < 5% mortality regardless of treatment. ** $p < 0.01$.

immune defense persist in wild type females for at least 24 hours after mating, and that mated females are compromised in their ability to induce expression of AMP genes after infection. Further, we demonstrate that seminal fluid elicits reduced overall defense against infection in the mated female, and that sperm and sex peptide play a crucial role in this effect. Finally, we demonstrate that females must have an intact germline in order for mating to drive any difference in overall immune defense. Taken together, these data indicate that reduction in systemic immune defense is a cost of reproduction in females, and that this cost is dependent on transfer of sperm and proteins in the seminal fluid. The immunological cost of mating could be direct, resulting for instance from genetic pleiotropy between egg production and immune signaling, or indirect, resulting from altered resource allocation after mating. Direct and indirect costs are not mutually exclusive, and disentangling them will require substantial additional experimentation.

The fact that a mating-induced reduction in female immune defense is not observed when the female lacks a germline or when the male fails to transfer sperm and seminal fluid proteins reveals that the effect of mating on immune defense is not simply due to wounding or general exertion associated with courting and the act of copulation. We previously reported that females vary genetically for the magnitude of post-mating immune depression they experience (Short and Lazzaro, 2010). Interestingly, however, males were genetically invariant for the degree of post-mating depression they elicit in their mates, despite the presently demonstrated dependence of the effect on male seminal fluid components (Short and Lazzaro, 2010). Considering this in the context of the data we present here, we suggest that mating results in a sustained shift in the female's physiology as she transitions from virgin homeostasis to active production of mature eggs, representing a genetically variable physiological trade-off between mating and immunity in females. We speculate that this could in part be mediated, for example, by a pleiotropic

signaling molecule such as juvenile hormone (Flatt et al., 2005). Notably, the production of juvenile hormone III-bisepoxide is stimulated *in vitro* by sex peptide (Moshitzky et al., 1996). Juvenile hormone plays an important role in controlling egg production (Soller et al., 1999) and reduces AMP gene expression in cell culture (Flatt et al., 2008), suggesting the hypothesis that juvenile hormone signaling might simultaneously contribute to the mating-induced reductions in overall defense and AMP gene expression we report in this work. We note that genetically variable physiological trade-offs like the one we describe here are likely to lie at the heart of evolutionary life history trade-offs (Flatt et al., 2005).

We analyzed humoral immune system activity during the course of infection in mated and virgin females and found that mated females exhibit lower AMP gene expression compared to virgin controls at four and twelve hours post-infection, despite mated females having equal and higher bacterial loads at these respective times. This finding is distinct from previously reported increases in AMP gene expression after mating in uninfected females (Fedorka et al., 2007; Innocenti and Morrow, 2009; Lawniczak and Begun, 2004; McGraw et al., 2004; Peng et al., 2005b; Wigby et al., 2008). These previous studies differed in design, from each other and from ours, perhaps accounting for the differences in effects seen between them. Most notably, females in our study were infected when AMP gene expression was assayed. It is possible that some of the increases in AMP gene expression reported by others may be tissue specific, and two studies have specifically identified changes in AMP gene expression in the reproductive tract after mating (Kapelnikov et al., 2008; Mack et al., 2006). AMP expression in the reproductive tract could be important for fighting local infection after mating. Our present data reveal a diminished capability of mated females to induce AMP genes in response to systemic infection as compared to the induction capability of virgin females. While the overall differences in AMP

expression due to mating are statistically significant, the actual fold differences between mating treatments for each gene are small (less than two-fold at each time point), and it is unclear whether they are sufficient to be solely responsible for the higher bacterial load and lower survival of mated females. We consider it possible that suppressed induction of the humoral immune system is only one of multiple mechanisms by which mating reduces female defense against infection.

Our data reveal that female flies are immunocompromised after mating only when they have intact germlines and when they receive sperm and accessory gland proteins from their mates. In particular, we observed the importance of one seminal fluid protein, sex peptide. Importantly, however, our data do not exclude the possibility that additional Acps may play a role, since neither sperm nor sex peptide individually account for the entire effect of mating on systemic bacterial load after infection.

The immune performance of eggless females is unaltered by mating, revealing an important role for the female germline. It is possible that the effect of mating on overall immune defense is due to post-mating changes in molecular or hormonal signaling that fail to occur in daughters of *tudor* females due to their absence of a germline. Another possibility is that the effect of mating on overall immune defense could be a consequence of producing mature eggs. The process of egg production is energetically demanding requiring females to synthesize large amounts of yolk protein, which is deposited into oocytes at the vitellogenic stages of oogenesis. Upon mating, transcription of yolk protein genes increases dramatically (Soller et al., 1997). The production of vitellogenic oocytes begins to increase in mated females at 6 hours after mating (Heifetz et al., 2001) and reaches very high levels by 14 hours after mating (Soller et al., 1997). This shift toward rapid and continuous egg production is arguably the most obvious and costly

effect that mating has on female physiology. Mating may induce changes in physiology or genetic signaling that act to prepare females for this long-term cost by altering utilization of resources to favor reproduction over defense.

In this context, we suggest that male delivery of sperm and SP may reduce female post-mating immune competence by inducing increases in egg production. In support of this hypothesis, we note that, in the first 24 hours after mating, females mated to spermless males have been shown to demonstrate significantly reduced levels of egg laying and fewer vitellogenic oocytes compared to females mated to wild type males (Heifetz et al., 2001). Additionally, in the first day post-mating, females mated to SP null or spermless/Acpless males have virgin-like levels of egg production and/or vitellogenesis (Heifetz et al., 2001; Kalb et al., 1993; Liu and Kubli, 2003). Over the next four days post-mating, females mated to spermless, spermless/Acpless or SP null males have been reported to lay eggs at virgin levels (Kalb et al., 1993; Liu and Kubli, 2003). Of note, our data are consistent with a model where sperm and SP may together contribute to a single effect on female post-mating immunocompetence (Peng et al., 2005a). Sex peptide is known to bind sperm tails and be slowly cleaved off over multiple days in the female sperm storage organs (Peng et al., 2005a). If long-term increases in egg production alter female immune defense, it is possible that sperm *per se* is not eliciting changes in female immune defense, but rather that it acts to facilitate the effect of SP by ensuring its stable storage in the female (Peng et al., 2005a). We note that alteration of immune defense due to long-term maintenance of SP signaling is unlikely to occur as a consequence of any direct effect of SP on JH signaling, as SP cleaved from sperm does not contain the N-terminus, which is crucial to elicit JH production (Fan et al., 2000). Unbound SP may act to alter immune defense

by affecting JH levels shortly after mating, while bound SP may have a later effect on defense by prolonging egg production.

Like Fedorka et al. (2007), we observed that females who did not produce late-stage oocytes failed to show reduced immune defense after mating. These results suggest that aspects of female physiology needed to produce mature oocytes - such as high-level production and secretion of yolk proteins, for example - intersect with immune defense ability. Moreover, it is interesting to consider the reasons why we observed no post-mating immune depression in germlineless females, whereas Fedorka et al. (2007) reported that ovo^{D1}mutant *Drosophila* females responded to mating with reduced immune defense until 9hrs. post-mating. Although the females in both studies were sterile and did not produce mature eggs, the cause of their sterility differs. The tudor-progeny females that we analyzed completely lack a germline and thus never initiate oogenesis (Boswell and Mahowald, 1985). In contrast, ovo^{D1} females initiate oogenesis, but arrest the process prior to the vitellogenic stages (Busson et al., 1983). Assuming that the difference in findings between the studies does not reflect genetic background differences between fly strains or procedural differences between the labs, they suggest that early post-mating effects on immune defense might be influenced by aspects of pre-vitellogenic signaling. Additional studies examining a range of female reproductive mutants that fail in various stages of egg development will help narrow down the specific aspects of oogenesis that are important for inhibiting immune defense.

5. Conclusions:

In summary, we report that reduced overall defense against infection suffered by *D. melanogaster* females after mating is not a result of the act of copulation, but is rather dependent on sperm and seminal fluid proteins, including sex peptide, transferred from males to females during mating. We also find that the effect is dependent on an intact female germline. We hypothesize that a physiological shift from virgin somatic homeostasis directly or indirectly compromises immune defense, including the ability to induce the humoral immune system. Such physiological trade-offs between mating and immune defense may reveal the mechanisms that underlie life history trade-offs and shape the evolution of both traits involved.

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Chapter 5.

Genome-wide gene expression analysis of response to infection in virgin versus mated female *Drosophila melanogaster.*

Abstract:

Copulation significantly reduces the ability to defend against systemic infection in female *Drosophila*, and the immunosuppressive effect of mating is contingent on the presence of a germline in females. We were interested in identifying genes that are likely to play a role in the reduced ability of mated females to defend themselves against infection. We used microarrays to compare genome-wide transcript levels in virgin females before and after infection to those of mated females before and after infection. We then repeated this entire experiment in female mutants that do not form a germline. We found that transcript levels of multiple genes involved in vitellogenesis are reduced in response to infection, but that this reduction is stronger in virgins than in mated females. These results are consistent with an interplay between egg production and immune defense. We also identified a number of immune responsive genes that are differentially induced after infection in virgins versus mated females. These genes are candidates to underlie the effect of mating on immune defense.

Introduction:

While our knowledge of the invertebrate immune system is extensive and continues to expand (reviewed in Wang and Ligoxygakis, 2006; Lemaitre and Hoffmann, 2007), our understanding of overall immune defense remains incomplete. Overall immune defense is defined as the combined abilities to immunologically eliminate pathogens and to tolerate the damage associated with an infection (Råberg et al., 2009). Part of our lack of understanding of immune defense stems from the fact that defense is not determined only by immune system activity but is also influenced by aspects of host physiology outside the canonical immune system. These non-immunological processes are often responsive to environmental factors such

as temperature, nutritional availability, or interactions with other organisms. The interconnection of defense with other diverse aspects of host physiology can set the stage for trade-offs between immunity and other costly life-history traits (Lazzaro and Little, 2009; Parker et al., 2011). Trade-offs between life-history traits and immunity have the potential to limit the evolution of immune efficacy, and their study forms the basis of the burgeoning field of ecological immunology (Sheldon and Verhulst, 1996; Siva-jothy et al., 2005; Schulenburg et al., 2009)

Historically, studies in this field have focused mainly on identifying trade-offs between immune defense and life history traits, yielding remarkable progress in our understanding of immune defense in an ecological and evolutionary context. Less emphasis has been placed on determining the mechanistic nature of these trade-offs, and our lack of mechanistic understanding represents a significant gap in our understanding of the function of immune defense (Schmid-Hempel, 2003). We and others have demonstrated that mated females suffer reduced ability to eliminate and survive pathogenic infection relative to virgin females (Fedorka et al., 2007; Short and Lazzaro, 2010). We have also shown that the effect of mating on immune defense is contingent on the proper formation of the female germline (Short et al., 2012), suggesting that post-mating immunosuppression is dependent on an as-yet unknown aspect of reproduction. The objective of the present study was to use transcriptional profiling to begin to identify why mated females demonstrate reduced immune defense. In order to address this question, we used whole-genome microarrays to test for differences in the transcriptional response of virgin females to systemic bacterial infection as compared to the response of mated females. We also sought to determine how infection status alters transcript levels of mating-responsive genes. Our goal was to identify genes that are most likely to be involved in shared

signaling between immunity and reproduction, and thus most likely to underlie the observed trade-off.

Methods:

Fly stocks and maintenance: Female flies used in this experiment were derived from two crosses: egg-producing females were *tud*^{1} *bw sp/*CS and were the daughters of a cross between *tud*^{1} *bw sp/*CyO mothers and Canton S fathers. Eggless females were also *tud*^{1} *bw sp/*CS but were the daughters of *tud*^{1} *bw sp* mothers and Canton S fathers. The mothers of the eggless females were homozygous for *tudor*^{1}, a maternal effect mutation that causes offspring to fail to form a germline. Egg-producing females had a genotype identical to eggless females but because their mothers were heterozygous for *tudor*^{1}, they produced normal numbers of eggs. Males used in mating experiments were from the standard laboratory strain Canton S.

Mating procedure: Eggless and egg-producing females were collected as virgins and aged for three days post-eclosion. The day before matings were to be set up, eggless and egg-producing females were lightly anaesthetized with CO_2 and put into individual vials with *ad libitum* access to food (8.3% glucose, 8.3% Brewer's yeast, and 1% agar, plus 0.04% phosphoric acid and 0.4% propionic acid added to inhibit microbial growth in the food). Females were randomly allocated to “virgin” or “mated” treatment groups and allowed to recover overnight. The following morning, within two hours of incubator “dawn,” a single male was aspirated into each vial containing a female assigned to the “mated” treatment and copulations were observed. Females from copulations lasting fewer than 15 minutes were discarded and not used for infections.

Infection procedure and sample preparation: 2.5 hours (±15minutes) after mating, mated eggless and egg-producing females were lightly anaesthetized with CO_2 and infected; virgin controls were infected in parallel. Infections were performed by dipping a 0.15mm anodized steel needle (Fine Science Tools, Inc.) into a dilute bacterial culture of the Gram-negative bacterial pathogen *Providencia rettgeri,* then piercing the thorax of the female fly. *P. rettgeri* was grown overnight in liquid LB at 37°C with shaking and diluted in sterile LB to an optical density of $A_{600} = 1.0 \pm 0.05$. In parallel, females to remain as uninjured controls were anaesthetized on CO_2 to control for effects of anesthesia. Infected mated and virgin females as well as uninjured virgin and mated controls were then put on fresh media in groups of approximately 10. A small number of flies were homogenized individually after each round of infection and an aliquot of undiluted homogenate was plated on LB agar using a spiral plater (Microbiology International) in order to estimate initial bacterial load. We found that our infection technique delivered an average dose of 10^3 bacteria to each female. Ten hours (±15 minutes) after infection (approximately 12.5 hours after mating), 25 whole female flies from each treatment were collected on CO_2, snap frozen in TRIZOL reagent (Ambion) and placed at -80°C. The entire experimental set up was replicated on three days, resulting in three biological replicates for each treatment.

RNA extraction and microarray preparation: We extracted RNA from our samples using TRIZOL reagent according to the manufacturer's protocol. Residual genomic DNA contamination was removed using TURBO DNA-*free* (Ambion) and the quality of the RNA from each sample was assessed using the BioAnalyzer 2100 (Agilent). Samples were labeled using Agilent's Low Input Quick Amp Labeling kit and were hybridized to 4x44K *Drosophila* (V2) Gene Expression Microarrays (Agilent) according to the manufacturer's instructions. RNA

labeling, microarray hybridizations and feature extraction were performed by the Cornell University Life Sciences Core Laboratory Center.

Microarray data analysis: The microarray data were analyzed using the Bioconductor package limma (Smyth, 2005). Data were background corrected using backgroundCorrect() and the "normexp" method recommended by Ritchie et al. (2007). We then normalized between all egg-producing arrays and between all eggless arrays using quantile normalization as recommended by Agilent and averaged signals between replicate probes. We generated lists of differentially expressed probesets utilizing the method for factorial designs outlined by Smyth (2005). We assayed for gene expression differences due to infection in both virgin and mated females as well as differences due to mating in both uninfected and infected females (Figure 5.1). These contrasts were initially performed within treatments of egg-producing females, and then were separately repeated for arrays from eggless females. We corrected for multiple tests using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995) with a false discovery rate of 5.0%. Finally, we validated the gene identities in our lists of differentially expressed probes and eliminated those that did not have an identifiable gene name or gene symbol on Flybase. Tests for enrichment of genes with related biological function were performed using GOrilla (Eden et al., 2009) to assign and analyze Biological Process Gene Ontology (GO) terms. REVIGO (Supek et al., 2011) was used to eliminate redundant GO terms and multiple-test correction for significant GO terms was performed using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995) with a false discovery rate of 5.0%.

Results and Discussion:

In this work, we were interested in identifying genes that may play a role in reducing immune defense in *D. melanogaster* females after mating. We infected mated, egg-producing females at 2.5 hours after the cessation of copulation alongside virgin, egg-producing controls. Ten hours after infection, we assayed genome-wide transcript abundance in infected virgin and mated females as well as in uninfected, age-matched virgin and mated females (Figure 5.1). All significant results for comparisons A through D from Figure 5.1 for egg-producing females can be found in Tables B1-B4. We replicated the entire experiment using females that genetically fail to form a germline in order to identify transcriptional differences that depend on germline development. All significant results for comparisons A through D for eggless females can be found in Tables B5-B8). We chose to assay transcript levels at 10 hours post-infection because mated females begin to demonstrate higher bacterial loads than virgins at approximately 12 hours post-infection (Figure B1, Short et al., 2012) and we were interested in identifying differences in transcript abundance that have the potential to contribute to this initial post-mating divergence in immune defense.

General expression response of egg-producing females after bacterial infection

After infection with the Gram-negative bacterial pathogen *Providencia rettgeri*, virgin and mated females shared many changes in gene expression (changes seen in both comparisons A and B in Figure 5.1). By examining these data, we could determine a general infection response profile of female *Drosophila melanogaster* that was consistent across different reproductive states. We detected significant expression changes in 168 probes as a result of bacterial infection, and these probes corresponded to 124 unique genes (Figure 5.2, Tables B1

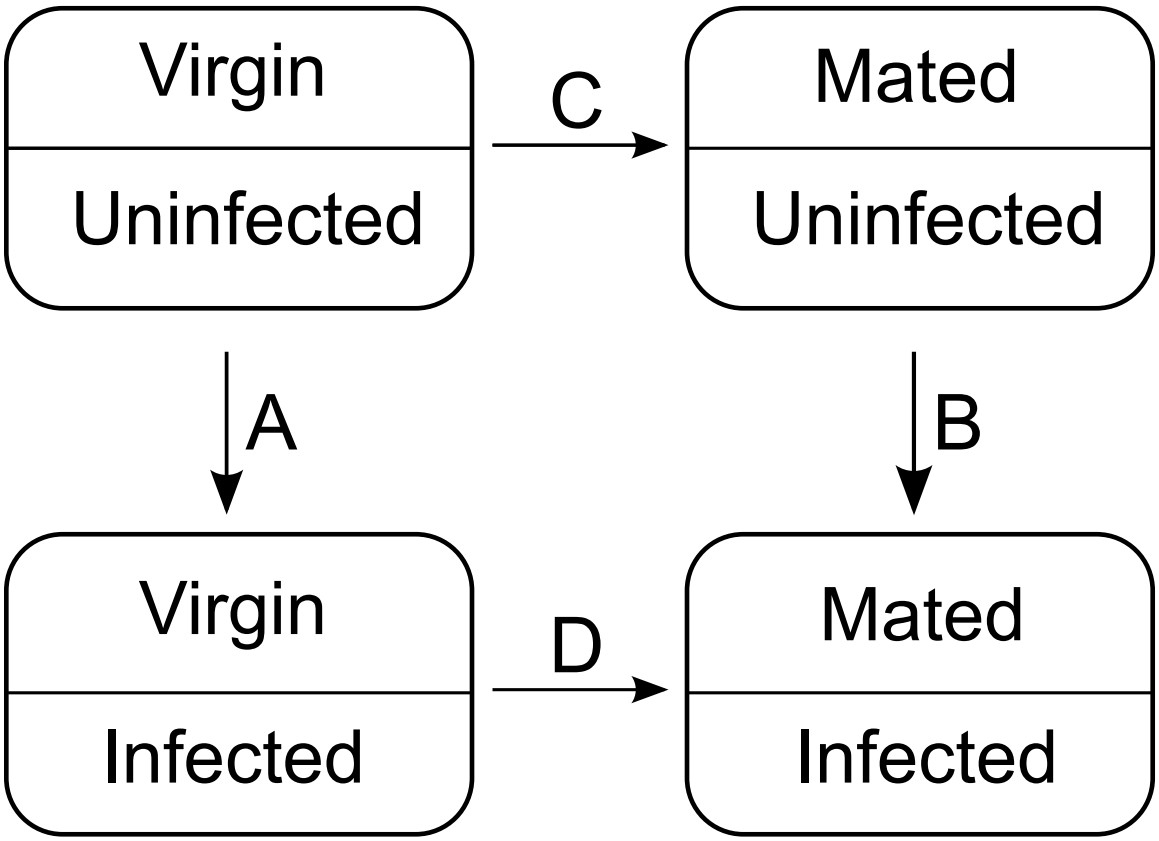


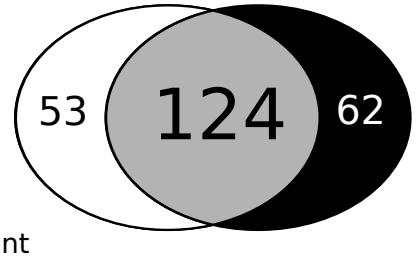
Figure 5.1: Experimental design. In order to determine ways in which immune defense and reproduction may be interacting to cause post-mating immunosuppression, we compared genome-wide transcript abundance between virgin and mated, infected and uninfected females. We assayed for differential transcript abundance between virgin uninfected females and virgin infected females (A) to identify infection-responsive genes in virgins, and performed the same analysis in mated females (B) to determine infection-responsive genes in mated females. By qualitatively comparing (A) to (B), we were able to establish differences in infection response that were dependent on mating status. By subtracting (A) from (B) we were able to ascertain which genes show the largest quantitative differences in infection response between virgin and mated females. We also assayed for differential transcript abundance in virgin uninfected females versus mated uninfected females (C), and between virgin infected females versus mated infected females (D) to determine which genes respond to mating and how that varies by infection state. We independently performed this entire experimental design in triplicate for both egg-producing females and eggless females.

Figure 5.2: The effect of infection on transcript abundance in virgin and mated females. We assayed for genes that demonstrated significant 2-fold or greater differences in transcript abundance in virgin, uninfected versus virgin, infected treatments and in mated, uninfected versus mated, infected treatments. We then determined which genes significantly change in transcript abundance due to infection in both virgin and mated females, only virgins or only mated females. Fold change values are in $\log_2$ units, and are expressed as uninfected minus infected signal, so a negative logFC represents an increase in signal in response to infection while a positive logFC represents a decrease in signal in response to infection. In instances where more than one probe showed significantly altered expression for a particular gene, only the probeset with the largest fold change is listed. GO term enrichment was determined using GOrilla and REVIGO was used to reduce lists of GO terms to those least redundant. Upward-pointing arrows indicate genes with increased expression and downward-pointing arrows indicate genes with depressed expression. A Benjamini-Hochberg correction (Benjamini and Hochberg, 1995) was performed to correct for multiple tests, and only GO terms that were significant after controlling for a false discovery rate of 5% were retained.

Both Virgin and Mated

103 - Immune response, stress response

21 - Vitelline membrane & chorion formation



Virgin Only

28 - no GO term enrichment

25 - Vitelline membrane & chorion formation

Up in response to infection			Down in response to infeciton		
Gene Symbol	logFC	BH adj. p-value	Gene Symbol	logFC	BH adj. p-value
CG11501	-4.91	0.02880	Vm32E	3.78	0.01686
CG43085	-2.10	0.00345	Vm34Ca	2.98	0.00808
Hsp70Bb	-2.02	0.03070	Vml	2.82	0.00059
CG13749	-1.48	0.00441	Vm26Ab	2.16	0.00125
CG15046	-1.47	0.00399	Lsp1beta	2.13	0.00145
Pu	-1.44	0.00074	to	2.09	0.00178
CG7367	-1.43	0.02175	fit	1.84	0.01160
CG30088	-1.42	0.00263	Try29F	1.58	0.04912
CG33459	-1.41	0.00042	CG43051	1.56	0.00035
CG33468	-1.38	0.00035	CG12398	1.49	0.01365
Ets21C	-1.33	0.00588	Obp99b	1.44	0.01356
CG8046	-1.33	0.02495	Jon99Ci	1.41	0.01365
CrebA	-1.33	0.00132	CG6704	1.40	0.04832
CG7442	-1.30	0.00034	CG10621	1.37	0.00117
CG14406	-1.30	0.02628	CG4830	1.36	0.02982
Esyt2	-1.27	0.00536	CG11854	1.35	0.00721
Cyp6w1	-1.25	0.00030	Odc1	1.32	0.00094
LpR2	-1.20	0.00025	CG3523	1.31	0.00165
CG9447	-1.17	0.00461	CG3348	1.24	0.03971
CG31664	-1.16	0.00036	CG1887	1.22	0.00131
lectin-24A	-1.13	0.02773	CG32425	1.04	0.00287
Pif1A	-1.13	0.00622	regucalcin	1.03	0.00151
CG14193	-1.09	0.01378	UGP	1.03	0.02335
CG15385	-1.08	0.00320	CG6067	1.02	0.00608
CG13795	-1.06	0.00512	CG15120	1.00	0.03714
Ddc	-1.02	0.03864			
RhoGAP18B	-1.02	0.01378			
Ect3	-1.01	0.00768			

Mated Only

36 - no GO term enrichment

26 - no GO term enrichment

Up in response to infection			Down in response to infection		
Gene Symbol	logFC	BH adj. p-value	Gene Symbol	logFC	BH adj. p-value
CG31775	-4.97	0.00011	Ir7c	2.07	0.00014
CG4757	-2.94	0.03909	CG17738	1.89	0.00097
IM23	-2.54	0.01761	CG34367	1.63	0.00161
Ugt37b1	-2.23	0.00091	lectin-28C	1.61	0.03026
TotX	-2.11	0.01828	CG31437	1.52	0.00081
CG9463	-1.97	0.00887	Vha16-2	1.52	0.00229
IM4	-1.51	0.00309	Damm	1.43	0.00918
CG13641	-1.48	0.03764	CG34247	1.38	0.00062
yellow-f	-1.47	0.00369	HLHm5	1.38	0.04980
Lip3	-1.45	0.01654	CG34136	1.33	0.00310
CG32023	-1.41	0.00372	CG34278	1.32	0.00525
CG15533	-1.41	0.00161	amd	1.26	0.04515
CG15065	-1.38	0.00988	alpha-Est2	1.23	0.00047
CG9780	-1.37	0.00013	HLHmgamma	1.23	0.00939
CG34291	-1.33	0.01650	GATAd	1.23	0.04310
CG6495	-1.33	0.00098	CG31778	1.21	0.00070
CG13311	-1.31	0.03963	CG17751	1.20	0.00022
CG9396	-1.29	0.00196	Tret1-2	1.17	0.00196
IM2	-1.28	0.04042	CG34205	1.12	0.00643
IM3	-1.23	0.00574	CG14095	1.11	0.00490
Smvt	-1.23	0.02711	CG3999	1.11	0.00378
CG16836	-1.22	0.00027	CG9747	1.07	0.00406
CG6553	-1.19	0.00979	shf	1.05	0.00068
CG34426	-1.15	0.00519	CG13042	1.02	0.03615
SerT	-1.13	0.00050	Orct	1.02	0.00043
CG7017	-1.13	0.00037	ndl	1.01	0.00054
CG5791	-1.12	0.00826			
CG4725	-1.09	0.00044			
CG8550	-1.08	0.00567			
Spn4	-1.06	0.00077			
CG9649	-1.06	0.00724			
hgo	-1.05	0.02117			
Spn1	-1.04	0.00176			
E5	-1.02	0.00724			
CG8449	-1.01	0.01482			
Spn28D	-1.00	0.02420			

and B2). Of the 124 genes whose expression changed in response to infection, 103 were upregulated. The vast majority of these genes are known immunity genes (Figure 5.2, Tables B1 and B2).

When we assigned GO terms to the genes upregulated after infection in both virgin and mated females, we found multiple GO terms relating to immune response and stress response were enriched (Table 5.1). Transcript abundance of antimicrobial peptide genes was dramatically increased due to infection (*CecA1, CecA2, CecB, AttA, AttB, AttC, AttD, Dpt, DptB, Mtk, Def, Dro, Drs, Drs-l,* Tables B1 and B2), as was that of many peptidoglycan recognition proteins (*PGRP-SA, PGRP-SB1, PGRP-SB2, PGRP-SC2, PGRP-SD, PGRP-LB, PGRP-LC PGRP-LF*, Tables B1 and B2). We also found infection-induced increases in transcript abundance in multiple genes in the *Turandot* gene family (*TotA, TotB, TotC and TotM*, Tables B1 and B2). At least one gene in the *Tot* family (*TotA*) and likely others are regulated by the JAK/STAT signaling pathway (Agaisse et al., 2003; Agaisse and Perrimon, 2004). Notably, Tot genes also respond to more general stress conditions (Ekengren and Hultmark, 2001), and they may alter immune defense through stress-response mechanisms such as tissue repair. Other upregulated genes that are known to play a role in immune defense included *TepII, IM3, IM1, IM10, Rel, pirk, spirit, edin, TsfI* and *nimB1* (Tables B1 and B2). We note that some of the genes we detected as being upregulated have negative regulatory roles in the humoral immune response (*PGRP-LB, PGRP-SC2, pirk*), illustrating mechanisms by which the host modulates immune system activity (Paredes et al., 2011).

Twenty-five probes corresponding to 21 unique genes showed reduced transcript abundance after infection in both virgins and mated females (Figure 5.2, Tables B1 and B2). Notably, this set of genes was enriched for genes involved in egg formation, specifically vitelline

Table 5.1: Biological process information for genes significantly altered by infection in virgin and/or mated Egg-producing females.

Gene list	GO term	GO term Description	Corrected p-value	# genes in GO category
Up significantly after infection in BOTH Virgin and Mated females	GO:0009617	response to bacterium	2.01E-38	31
	GO:0006952	defense response	2.65E-37	35
	GO:0009607	response to biotic stimulus	5.01E-34	32
	GO:0051704	multi-organism process	7.76E-30	33
	GO:0006955	immune response	5.07E-29	28
	GO:0002376	immune system process	4.03E-27	28
	GO:0006950	response to stress	4.96E-26	41
	GO:0009253	peptidoglycan catabolic process	8.26E-12	8
	GO:0050896	response to stimulus	2.91E-11	45
	GO:0030203	glycosaminoglycan metabolic process	3.23E-09	8
	GO:0016052	carbohydrate catabolic process	5.56E-07	9
	GO:0005976	polysaccharide metabolic process	1.71E-05	10
	GO:0031347	regulation of defense response	8.52E-05	6
	GO:0034605	cellular response to heat	9.98E-05	5
	GO:0009308	amine metabolic process	5.53E-04	12
	GO:0005975	carbohydrate metabolic process	6.19E-04	13
	GO:0043900	regulation of multi-organism process	6.32E-04	6
	GO:0009595	detection of biotic stimulus	1.22E-03	3
	GO:0009057	macromolecule catabolic process	1.91E-03	9
	GO:0080134	regulation of response to stress	3.45E-03	6
	GO:0034644	cellular response to UV	8.01E-03	3
	GO:0008063	Toll signaling pathway	1.29E-02	4
	GO:0009266	response to temperature stimulus	1.39E-02	6
	GO:0061060	negative regulation of peptidoglycan recognition protein signaling pathway	1.81E-02	2
	GO:0071214	cellular response to abiotic stimulus	3.19E-02	3
	GO:0009411	response to UV	3.67E-02	3
Down significantly after infection in BOTH Virgin and Mated females	GO:0007305	vitelline membrane formation involved in chorion-containing eggshell formation	1.04E-03	3
	GO:0022412	cellular process involved in reproduction in multicellular organism	1.63E-03	4
	GO:0010927	cellular component assembly involved in morphogenesis	3.51E-03	4
	GO:0043062	extracellular structure organization	1.75E-02	3
Up significantly after infection in ONLY Virgin females	No enrichment			
Down significantly after infection in ONLY Virgin females	GO:0007305	vitelline membrane formation involved in chorion-containing eggshell formation	1.75E-05	4
	GO:0043062	extracellular structure organization	9.23E-04	4
	GO:0022412	cellular process involved in reproduction in multicellular organism	1.08E-03	4
	GO:0010927	cellular component assembly involved in morphogenesis	1.02E-02	4
Up significantly after infection in ONLY Mated females	No enrichment			
Down significantly after infection in ONLY Mated females	No enrichment			

membrane and chorion formation (*Vm26Ac, Vml, psd and dec-1,* Table 5.1, Tables B1 and B2). This result is particularly compelling given that female *D. melanogaster* suffer a germline-dependent reduction in immune defense after mating (Short et al., 2012). A generalized decrease in transcription of genes crucial for oogenesis is consistent with a scenario in which reproduction and immune defense are physiologically in conflict.

The effect of mating status on infection response in egg-producing females

We and others have shown that mated females suffer reduced ability to defend against systemic infection relative to virgin females (Fedorka et al., 2007; Short and Lazzaro, 2010; Short et al., 2012). We hypothesized that virgin females may exhibit gene expression differences after infection that differ from those of mated females, which could inform the nature of the physiological trade-off we have observed between reproduction and immune defense.

We determined that 61 probesets corresponding to 53 unique genes were significantly affected by infection in virgin (comparison A in Figure 5.1) but not mated females (comparison B in Figure 5.1; Figure 5.2). Of these 53 genes, 28 of them were upregulated by infection while 25 of them were downregulated. GO analysis on the unique genes corresponding to upregulated probesets revealed no enrichment of particular biological processes (Figure 5.2, Table 5.1). However, genes involved in vitelline membrane and egg coat formation were enriched within the group of downregulated genes (Figure 5.2, Table 5.1). This enrichment was primarily due to virgin-specific reductions in transcript abundance for the genes *Vm32E* (down 13.74 fold), *Vm34Ca* (down a maximum of 7.89 fold), *Vml* (down 7.06 fold) and *Vm26Ab* (down 4.47 fold) (Figure 5.2, Table B1). These data suggest that non-reproductive (*i.e.* virgin) females preferentially suppress expression of genes in egg formation when faced with systemic bacterial

infection. These genes are not significantly affected by infection in mated females, likely because mated females continue to produce mature eggs even while combating infection.

We performed reciprocal analysis to identify changes in gene expression in response to infection that were only significant in mated females but not in virgins (significant in comparison B but not in comparison A in Figure 5.1). We found 72 probesets corresponding to 62 unique genes that were significantly altered by infection in mated females only (Figure 5.2). Of these 62 genes, 36 were upregulated by infection while 26 were downregulated (Figure 5.2). We found no GO categories enriched within either the upregulated or downregulated genes, or in the entire set of 62 genes (Table 5.1). Nonetheless, we note that expression of some immune-annotated genes were significantly increased in response to infection in mated females but not in virgins, including *IM2, IM3, IM4, IM23, TotX,* and *yellow-f* (Figure 5.2). This was somewhat surprising given that mated females have lower immune defense than virgin females. At 10 hours post-infection, when we assayed gene expression, mated females do not have higher levels of bacteria than virgin controls (Figure S1), so we think it is unlikely that this higher immune gene transcript abundance reflects increased positive stimulation of the immune system through higher pathogen load.

In addition to querying probesets that were significantly altered by infection in one mating status but not the other, we were also interested in identifying probesets that differed quantitatively in the degree to which expression changed between virgin and mated females. We assessed this by identifying genes for which the absolute value of (Comparison A – Comparison B) was greater than 1.0, indicating at least a 2 fold difference in response to infection in virgins versus mated females (Table B9). There were 335 unique genes that met this criterion. We found that for 68 of these genes, the virgin response to infection was significantly different from the

mated response to infection at a nominal (uncorrected) p-value of 0.05 (Table B9). GO analysis of the 335 genes showed significant enrichment for four Biological Process terms: "defense response to gram-positive bacterium," "defense response," "ATP biosynthetic process" and "vitelline membrane formation involved in chorion-containing eggshell formation" (Table 5.2).

Multiple genes implicated in immune defense were differentially affected by infection in virgins compared to mated females (Table 5.2). The transcript level of *TepII* is significantly higher after infection in virgins relative to mated females ($p < 0.05$, Table B9). All of the *Attacin* genes and *TotM* are also more strongly induced in virgin females relative to mated females, although not significantly so (Table B9). *PGRP-SD* and *IM4* show significantly higher expression in mated females than in virgins ($p < 0.05$ in both cases, Table B9), while *sphinx2, r2d2* and *Gr28b* are increased in response to infection in mated females but decreased in virgins (*r2d2* $p < 0.05$, Table B9). These data reveal that virgins respond differently to infection than do mated females, although the differences are complex. The *Attacin* genes and *TepII*, which are induced to a greater degree in virgins, are directly involved in bacterial elimination. *PGRP-SD*, which is induced to a greater degree in mated females, is best characterized as encoding a protein that recognizes Gram-positive bacterial infection (Bischoff et al., 2004; Wang et al., 2008). *IM4* is induced in response to bacterial infection and its transcription depends on the same signaling pathways that regulate antimicrobial peptide gene expression (Uttenweiler-Joseph et al., 1998), but the function of IM4 protein is unknown. *Sphinx2* is a serine protease homolog and a paralog of *sphinx1.* Toll immune signaling is strongly reduced when both *sphinx1* and *sphinx2* are simultaneously knocked down using RNAi, but it is not yet clear whether *sphinx2* has an effect on immunity independent of *sphinx1* (Kambris et al., 2006). *R2d2* is part of the RNA

Table 5.2: Biological process information for genes showing change in transcript levels due to infection that differs by 2-fold or more in virgin versus mated egg-producing females

GO term	GO term Description	Corrected p-value	Genes in GO category
GO:0007305	vitelline membrane formation involved in chorion-containing eggshell formation	0.000403	Vm26Aa Vm26Ab Vml Vm34Ca Vm32E closca
GO:0050830	defense response to Gram-positive bacterium	0.00281968	sphinx2 AttA AttB AttC AttD PGRP-SD TotM CG30098
GO:0006754	ATP biosynthetic process	0.02630268	Ca-P60A CG17300 CG5389 ATPsyn-gamma CG12027 ATP7
GO:0006952	defense response	0.04510244	sphinx2 IM4 r2d2 CG30098 PGRP-SD AttA AttB AttC AttD Gr28b TepII Eig71Eg TotM Tsf1

interference machinery of *Drosophila* and plays an important role in antiviral immunity but not antibacterial immunity, and given that we performed infections with a bacterial pathogen, the implications of this result are unclear (Wang et al., 2006). *Gr28b* is involved in immune defense (Ayres et al., 2008) but this is likely due to its role in regulating feeding behavior, which also alters defense against certain bacterial pathogens (Ayres and Schneider, 2009).

The GO category "ATP biosynthetic process" contained genes encoding proteins with multiple roles in basic metabolic processes, such as ATP synthesis (*ATPsyn-gamma*) and ion transport (*Ca-P60A, ATP7*) (Table 5.2, Table B9). It is not clear why expression of these genes is differentially affected by infection in virgins versus mated females, though it does suggest that basic metabolic functions may be differentially affected by infection depending on mating status.

Our list of genes showing differential expression in virgin versus mated females after infection also included a number of vitelline membrane formation genes: *Vm26Aa, Vm26Ab, Vm34Ca, Vm32E, Vml* and *closca* (Table 5.2, Table B9). Of these, *Vml, Vm26Ab and Vm34Ca* were all nominally significant ($p < 0.05$, Table B9). For all six vitelline membrane genes (the five above plus *Vm26Ac*), transcript abundance was higher in mated females compared to virgins, which is expected given that mated females actively produce higher numbers of eggs (Figure 5.3). We also found that, for all six genes, transcript abundance was reduced in response to infection in both mated and virgin females which is consistent with a physiological trade-off between immune defense and reproduction (Figure 5.3). This reduction was much more extreme in virgin females than in mated females in five out of six genes (Figure 5.3), which suggests that virgin females may improve their immune defense by withdrawing resources that would otherwise be spent on reproduction, whereas mated females may not have that option.

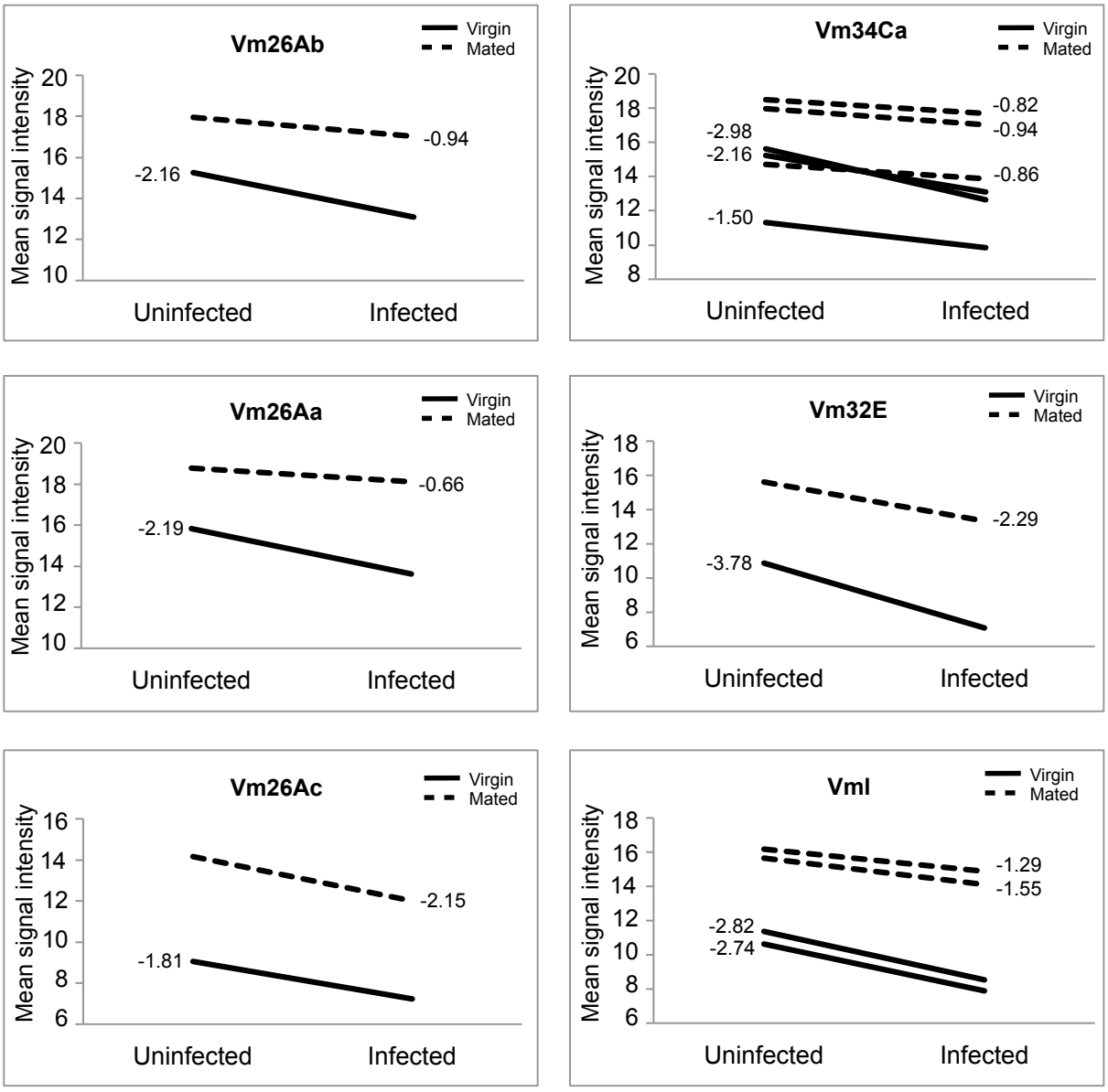


Figure 5.3: Vitelline membrane gene transcript abundance in egg-producing females. For all probesets that mapped to vitelline membrane genes, we determined averaged normalized signal intensity across all three biological replicates for each treatment. Only a single probeset exists on the array for Vm26Aa, Vm26Ab, Vm26Ac and Vm32E, but Vm34Ca has three probesets and Vml has two. We then determined the change in mean signal intensity due to infection for virgin and mated females. These values are plotted to the left of each virgin line (solid) and to the right of each mated line (dashed) for each gene.

Vitelline membrane proteins are secreted during stages 8-10 of oogenesis by somatic follicle cells that surround the oocyte (Burke et al., 1987; Gigliotti et al., 1989). They form the vitelline membrane, the innermost layer of the *Drosophila* eggshell (Margaritis et al., 1980). It is not clear from these data why vitelline membrane gene transcripts decreased in abundance in response to infection, nor is it clear why this phenomenon was more pronounced in virgin females relative to mated females. It is tempting to speculate that virgins may slow or alter oocyte progression when infected in a way that improves their ability to fight infection, and that it may be maladaptive or physiologically impossible for mated females to do the same. This infection-induced reduction in vitelline membrane transcripts could be the indirect result of a reallocation of resources toward immune defense and away from reproduction, or it may be the result of antagonistic signaling between the immune system and egg production. However, the nature of any interaction between vitelline membrane gene expression and immune defense, whether direct or indirect, will require further investigation.

The effect of infection on mating-responsive genes in egg-producing females

Given that mated females suffer reduced systemic immune defense relative to virgins (Fedorka et al., 2007; Short and Lazzaro, 2010), we were interested in identifying changes in gene expression that occur with mating in uninfected (comparison C in Figure 5.1, Table B3) and/or infected females (comparison D in Figure 5.1, Table B4). Multiple microarray studies have investigated differences in transcript abundance due to mating in females outside the context of infection (*e.g.* Lawniczak and Begun, 2004; McGraw et al., 2004; Innocenti and Morrow, 2009). They reported upregulation of multiple immunity genes in response to mating, and increases in baseline expression of antimicrobial peptide genes could potentially confer

increased protection against infection. This result is seemingly counterintuitive given that mated females perform more poorly than virgins in response to systemic infection by *P. rettgeri*. However, all females used in these previous studies were uninfected. We specifically measured mating-induced changes in infected flies in addition to uninfected flies because we hypothesized that an ongoing infection may alter the female's capacity to initiate her reproductive programme.

In our study, females were assayed at 12.5 hours after mating cessation. There were 1390 probes corresponding to 1137 unique genes that were significantly altered by mating in one or both infection states (comparison C and/or D in Figure 5.1, Figure 5.4). There were 489 unique genes whose expression was altered by mating in both uninfected (comparison C in Figure 5.1) and infected females (comparison D in Figure 5.1, Tables B3 and B4). Of these, 286 genes were significantly upregulated in both uninfected and infected females and 203 genes were significantly downregulated in both treatments (Figure 5.4, Tables B3 and B4). A large number of genes were specifically altered in either uninfected or infected females. There were 101 genes significantly upregulated and 101 genes significantly downregulated after mating in uninfected females, but mating did not significantly alter the expression of these 202 genes in infected females (Figure 5.4, Table B3). Reciprocally, there were 225 genes that were upregulated and 288 genes downregulated in response to mating in infected females only (Figure 5.4, Table B4).

We assigned GO terms to the genes whose expression was significantly altered by mating in the uninfected and/or infected females and tested for enrichment of specific terms (Table 5.3). Among the genes with increased expression in both uninfected and infected females, we found enrichment of transcripts that function in proteolysis and formation of the vitelline membrane. This is expected given that vitelline membrane genes are highly expressed during the vitellogenic stages of oogenesis (Stages 8-10, Burke et al., 1987; Gigliotti et al., 1989), and

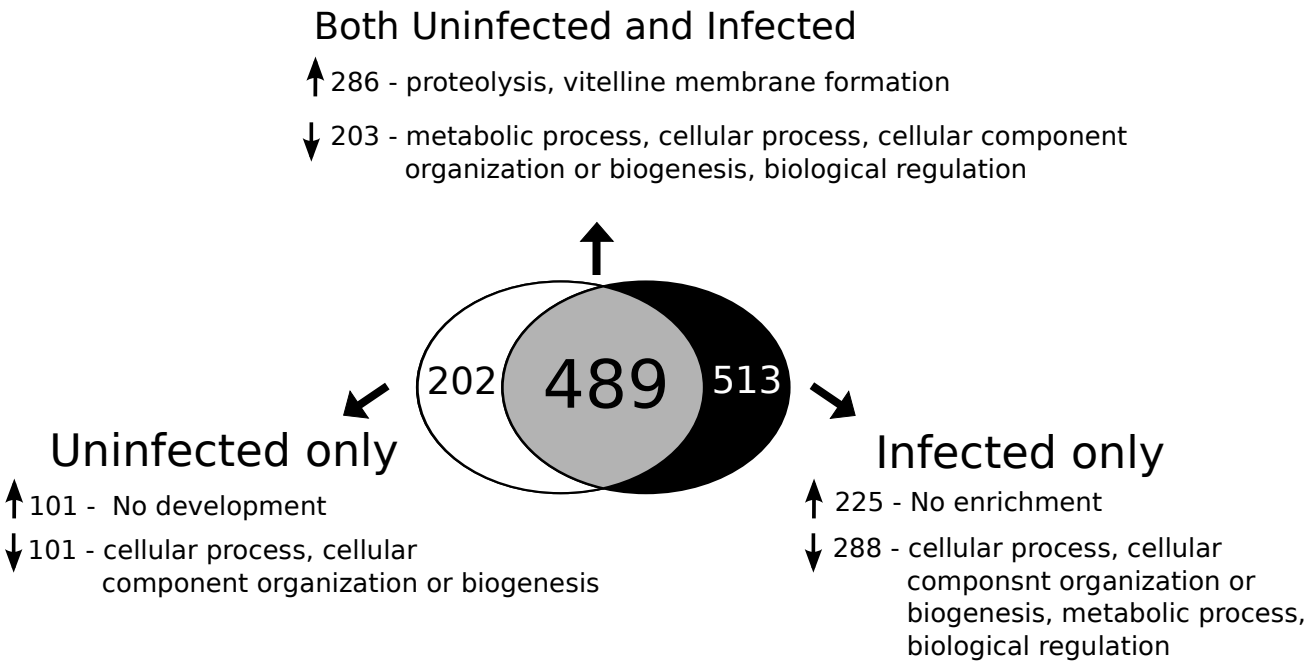


Figure 5.4: The effect of mating on transcript abundance in uninfected and infected females. We assayed for genes that showed significant 2-fold or greater differences in transcript abundance in virgin, uninfected versus mated, uninfected treatments and in virgin, infected versus mated, infected treatments. We then determined which genes significantly change in transcript abundance due to mating in both uninfected and infected females, only uninfected or only infected females. GO term enrichment was determined for each set of genes using GOrilla, and REVIGO was used to reduce lists of GO terms to those least redundant. Upward-pointing arrows indicate genes with increased expression and downward-pointing arrows indicate genes with depressed expression. A Benjamini-Hochberg correction (Benjamini and Hochberg, 1995) was performed to correct for multiple tests, and only GO terms that were significant after controlling for a false discovery rate of 5% were retained.

Table 5.3: Biological process information for genes significantly altered by mating in uninfected and/or infected Egg-producing females.

Gene list	GO term	GO term Description	Corrected p-value	# genes in GO category
Up significantly after mating in BOTH Uninfected and Infected females	GO:0006508	proteolysis	2.41E-13	51
	GO:0007305	vitelline membrane formation involved in chorion-containing eggshell formation	4.93E-08	8
	GO:0043062	extracellular structure organization	6.61E-05	9
	GO:0022412	cellular process involved in reproduction in multicellular organism	5.62E-06	10
	GO:0010927	cellular component assembly involved in morphogenesis	1.92E-03	10
Down significantly after mating in BOTH Uninfected and Infected females	GO:0006259	DNA metabolic process	7.71E-14	28
	GO:0007051	spindle organization	3.15E-08	21
	GO:0006996	organelle organization	1.30E-06	42
	GO:0051276	chromosome organization	1.70E-06	21
	GO:0007059	chromosome segregation	1.92E-06	13
	GO:0090304	nucleic acid metabolic process	4.76E-05	34
	GO:0006260	DNA replication	4.89E-05	10
	GO:0010564	regulation of cell cycle process	4.92E-05	15
	GO:0006139	nucleobase-containing compound metabolic process	5.02E-05	39
	GO:0007010	cytoskeleton organization	5.15E-05	25
	GO:0007017	microtubule-based process	8.83E-05	23
	GO:0051726	regulation of cell cycle	1.81E-04	17
	GO:0006270	DNA-dependent DNA replication initiation	1.92E-04	5
	GO:0034641	cellular nitrogen compound metabolic process	1.92E-04	41
	GO:0006310	DNA recombination	6.89E-04	7
	GO:0051382	kinetochore assembly	8.62E-04	3
	GO:0009132	nucleoside diphosphate metabolic process	1.41E-03	4
	GO:0009220	pyrimidine ribonucleotide biosynthetic process	1.51E-03	4
	GO:0070925	organelle assembly	1.67E-03	7
	GO:0006807	nitrogen compound metabolic process	1.96E-03	41
	GO:0009949	polarity specification of anterior/posterior axis	5.18E-03	3
	GO:0071840	cellular component organization or biogenesis	5.19E-03	46
	GO:0051313	attachment of spindle microtubules to chromosome	5.32E-03	3
	GO:0006165	nucleoside diphosphate phosphorylation	5.77E-03	3
	GO:0051383	kinetochore organization	8.49E-03	3
	GO:0065003	macromolecular complex assembly	8.56E-03	11
	GO:0065001	specification of axis polarity	8.68E-03	3
	GO:0006333	chromatin assembly or disassembly	8.68E-03	5
	GO:0051303	establishment of chromosome localization	1.20E-02	4
	GO:0046939	nucleotide phosphorylation	1.85E-02	3
	GO:0072527	pyrimidine-containing compound metabolic process	2.09E-02	4
	GO:0006974	response to DNA damage stimulus	2.28E-02	12
	GO:0045035	sensory organ precursor cell division	2.31E-02	3
	GO:0000910	cytokinesis	2.33E-02	7
	GO:0033043	regulation of organelle organization	2.48E-02	9
	GO:0001709	cell fate determination	3.10E-02	8
	GO:0043933	macromolecular complex subunit organization	3.12E-02	11
	GO:0009994	oocyte differentiation	3.13E-02	3
	GO:0044260	cellular macromolecule metabolic process	3.32E-02	47
	GO:0001964	startle response	4.86E-02	3
Up significantly after mating in ONLY Uninfected females	No enrichment			
Down significantly after mating in ONLY Uninfected females	GO:0051276	chromosome organization	6.78E-11	20
	GO:0006325	chromatin organization	1.61E-10	16
	GO:0034728	nucleosome organization	1.37E-07	9
	GO:0065004	protein-DNA complex assembly	2.24E-07	9
	GO:0071824	protein-DNA complex subunit organization	3.61E-07	9

	GO:0006996	organelle organization	5.52E-06	26
	GO:0043933	macromolecular complex subunit organization	3.05E-05	12
	GO:0071840	cellular component organization or biogenesis	4.71E-04	29
	GO:0007059	chromosome segregation	2.06E-03	7
	GO:0048869	cellular developmental process	9.17E-03	22
	GO:0006259	DNA metabolic process	1.60E-02	9
	GO:0071844	cellular component assembly at cellular level	1.62E-02	12
	GO:0007049	cell cycle	2.16E-02	6
	GO:0051726	regulation of cell cycle	2.99E-02	9
	GO:0000082	G1/S transition of mitotic cell cycle	3.05E-02	3
	GO:0051310	metaphase plate congression	3.65E-02	3
	GO:0030154	cell differentiation	3.73E-02	15
Up significantly after mating in ONLY Infected females	No enrichment			
Down significantly after mating in ONLY Infected females	GO:0051276	chromosome organization	1.83E-04	22
	GO:0007346	regulation of mitotic cell cycle	4.29E-04	17
	GO:0051726	regulation of cell cycle	6.68E-04	20
	GO:0006259	DNA metabolic process	1.68E-03	18
	GO:0045596	negative regulation of cell differentiation	6.94E-03	11
	GO:0050794	regulation of cellular process	8.05E-03	78
	GO:0006281	DNA repair	8.25E-03	10
	GO:0009794	regulation of mitotic cell cycle, embryonic	8.78E-03	4
	GO:0065007	biological regulation	9.06E-03	88
	GO:0050789	regulation of biological process	1.01E-02	82
	GO:0007059	chromosome segregation	1.05E-02	10
	GO:0044260	cellular macromolecule metabolic process	1.09E-02	64
	GO:0006996	organelle organization	1.18E-02	40
	GO:0010468	regulation of gene expression	1.64E-02	38
	GO:0019222	regulation of metabolic process	1.90E-02	45
	GO:0032880	regulation of protein localization	2.15E-02	7
	GO:0051093	negative regulation of developmental process	2.22E-02	11
	GO:0048519	negative regulation of biological process	2.51E-02	31
	GO:0050793	regulation of developmental process	3.48E-02	21
	GO:0006464	protein modification process	3.99E-02	28
	GO:0048523	negative regulation of cellular process	4.10E-02	27
	GO:0045132	meiotic chromosome segregation	4.14E-02	6
	GO:0043412	macromolecule modification	4.16E-02	29
	GO:0051017	actin filament bundle assembly	4.38E-02	4
	GO:0042683	negative regulation of compound eye cone cell fate specification	4.40E-02	2
	GO:0051301	cell division	4.41E-02	9
	GO:0006325	chromatin organization	4.42E-02	11
	GO:0043161	proteasomal ubiquitin-dependent protein catabolic process	4.53E-02	4
	GO:0006348	chromatin silencing at telomere	4.58E-02	2
	GO:0090068	positive regulation of cell cycle process	4.64E-02	4
	GO:0045995	regulation of embryonic development	4.72E-02	7
	GO:0071840	cellular component organization or biogenesis	4.89E-02	53

mated females are actively producing high numbers of vitellogenic oocytes at ~12 hours post-mating when these measurements were taken (Heifetz et al., 2001).

Genes encoding proteolysis regulators could be involved in many possible post-mating functions, including the processing of seminal fluid proteins (*e.g.* Pilpel et al., 2008). Proteolysis-regulator encoding genes also function in immunity, and act to regulate melanization and humoral immune signaling (Cerenius and Söderhäll, 2004; Wang and Ligoxygakis, 2006). Many of the proteolysis genes we detected as being upregulated by mating belong to the *Jonah* gene family (*Jon65Aii, Jon65Aiii, Jon65Aiv, Jon25Bi, Jon25Bii, Jon99Cii, Jon44E, Jon74E, Jon99Fi, Jon99Fii and Jon66Ci*, Tables B3 and B4). *Jonah* genes have previously been reported to be expressed only in the midgut (Akam and Carlson, 1985). *Jonah* genes are downregulated in response to infection (this study: *Jon99Fi* and *Jon99Ci*, Tables B1 and B2; De Gregorio et al., 2001: *Jon44E, Jon25Bi, JonBii, Jon99Fi*). The induction of *Jonah* genes by mating and their repression by infection may indicate one antagonistic pleiotropy between immunity and reproduction.

Genes with reduced transcript abundance after mating in both uninfected and infected females were enriched for many GO terms involved in cellular replication, including chromosome segregation, regulation of cell cycle, DNA replication and spindle organization (Table 5.3). These and other related GO categories were also enriched among genes whose expression is repressed by mating specifically in uninfected females or specifically in infected females. It initially surprised us that these transcripts were reduced in abundance given that oocyte production, which increases after mating, requires cell division and reorganization. However, mated females lay a large number of mature eggs shortly after mating, and because of this have fewer late-stage oocytes (stages 13-14) than virgins at the time of our assay (Heifetz et

al., 2001). We hypothesized that many of these transcripts may actually be maternally deposited into late-stage oocytes and the reduction in the transcript level of these genes may merely reflect the fact that the late-stage oocytes bearing these transcripts have already begun to be laid by mated females. To test this, we compared our list of downregulated genes to two independently generated lists of maternal transcripts (Hooper et al., 2007; The Berkeley Drosophila Genome Project, Tomancak et al., 2002, 2007) and found that 62.1% of the genes reduced due to mating in both uninfected and infected females have been identified as being maternally deposited into oocytes. Similarly, 61.5% of the genes whose transcript abundance was significantly reduced only in infected females and 42.6% of those reduced only in uninfected females are maternally deposited. While this does not account for all of the genes showing reduced expression after mating in uninfected and/or infected females, we think that maternal deposition of transcripts into oocytes probably accounts for much of the observed result.

While uninfected and infected females demonstrated generally similar patterns of change in transcript abundance after mating (Table 5.3), we note that the GO term "humoral immune response" (GO:0006959) was enriched among genes that showed increased transcript abundance after mating specifically in infected females, but it did not survive correction for multiple testing ($p = 6.36 \times 10^{-5}$, corrected p-value = 0.102, data not shown). Because our multiple testing correction was rather stringent, we felt that this result warranted further investigation. This GO term included two immune induced molecules (*IM4* and *IM10*) and five genes with lysozyme activity (*LysB, LysC, LysD, LysE* and *CG16799*) whose expression was significantly higher after mating in infected females but not in uninfected females (Table B4). The lysozyme genes upregulated in response to mating comprise the *LysD*-like gene family, which is thought to be expressed only in the gut of adult flies (Daffre et al., 1994). It is possible that this result is related

to infection-induced changes in the gut rather than being a direct result of systemic infection. Mating has been shown to increase food intake (Carvalho et al., 2006), and these gut-specific mating-induced changes in gene expression may be a result of altered feeding behavior. However, it is unclear why immunity genes with gut restricted expression would respond to mating in an infection dependent manner.

Infection does little to alter mating-induced changes in transcript abundance in eggless females

We have previously shown that post-mating depression in overall immune defense of *D. melanogaster* females is dependent on the formation of an intact germline (Short et al., 2012). We therefore sought to determine whether females that lack a germline show an altered transcriptional response to mating and infection compared to females with intact germlines. Our germline-less females are daughters of *tudor* mothers (see Methods).

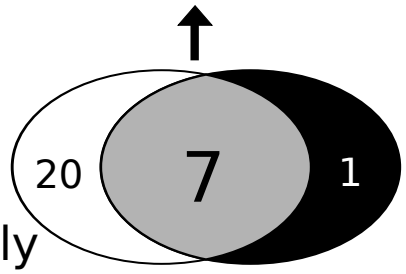
Mating itself induced very few transcriptional changes in eggless females. Only seven genes were altered after mating in both uninfected and infected eggless females (Figure 5.5). One of these genes was *Jon25Bi*, suggesting that the post-mating change in transcription of *Jonah* genes by egg-producing females is at least partly independent of the presence of a germline. Uninfected females exhibited increases in transcript abundance of genes enriched for mannose metabolism after mating, a result that was not observed in infected females after mating (GO term p-value = 7.52×10^{-4}; Figure 5.5). It is possible that this may be indicative of germline-independent mating-induced changes in metabolism that fail to occur when the female is infected, though more data are needed to develop this interpretation beyond speculation.

Both Uninfected and Infected

4 - no GO term enrichment

3 - no GO term enrichment

Gene Symbol	Infected logFC	BH adj. p-value	Uninfected logFC	BH adj. p-value
CG13078	-1.01	0.03392	-1.35	0.00226
CG17192	-1.44	0.02864	-1.42	0.00789
CG32751	-1.43	0.02152	-1.89	0.00178
Jon25Bi	-1.21	0.03392	-1.15	0.01076
CG15263	1.63	0.01037	1.59	0.00342
CG34040	1.58	0.00476	1.92	0.00102
Send1	1.79	0.00476	1.69	0.00226



Uninfected Only

12 - Mannose metabolic process

8 - no GO term enrichment

Up in response to mating

Gene Symbol	logFC	BH adj. p-value
CG4830	-2.31	0.00226
CG17192	-1.59	0.01012
CG9465	-1.58	0.01266
CG9463	-1.50	0.00672
CG13078	-1.40	0.00178
CG11912	-1.21	0.00054
ninaD	-1.19	0.04433
CG16743	-1.15	0.00178
Npc2d	-1.08	0.00305
CG10592	-1.06	0.02397
CG9466	-1.04	0.02333
tun	-1.01	0.01900

Down in response to mating

Gene Symbol	logFC	BH adj. p-value
Try29F	3.20	0.00214
CG13091	1.87	0.04867
Mal-B1	1.61	0.00695
CG30281	1.34	0.02764
tobi	1.12	0.01400
Prat2	1.11	0.01266
CG6012	1.04	0.00269
CG15199	1.03	0.00316

Infected Only

1 - no GO term enrichment

0 - no GO term enrichment

Up in response to mating

Gene Symbol	logFC	BH adj. p-value
CG31775	-1.64	0.03392

Down in response to mating

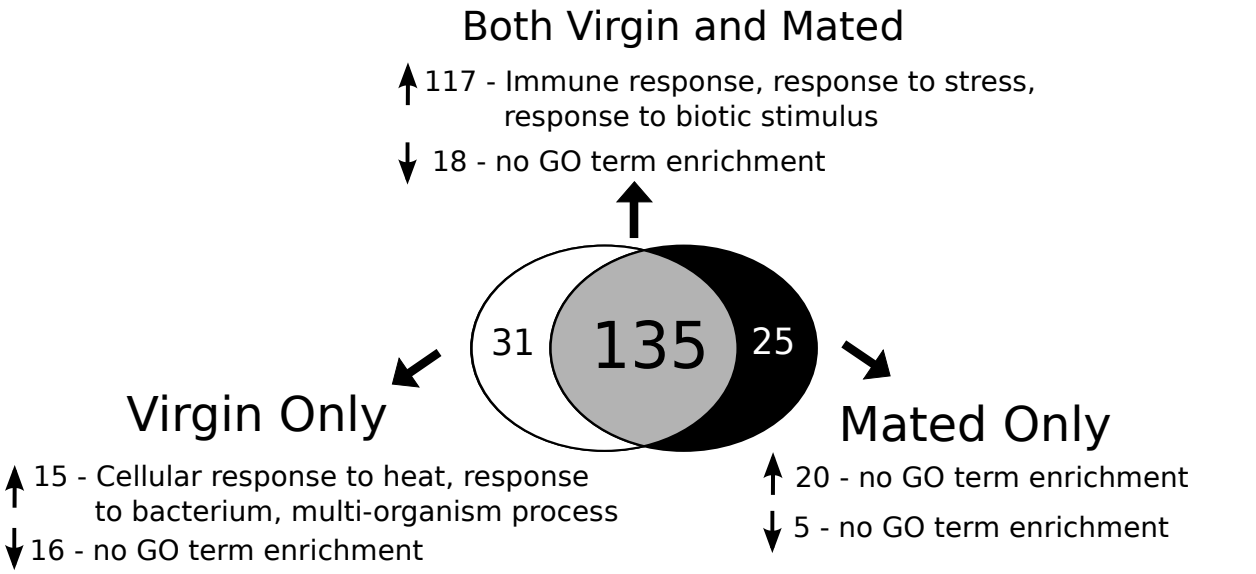
None

Figure 5.5: The effect of mating on transcript abundance in uninfected and infected eggless females. We assayed for genes that showed significant 2-fold or greater differences in expression in virgin, uninfected versus mated, uninfected treatments and in virgin, infected versus mated, infected treatments. We then determined which genes significantly change in transcript abundance due to mating in both uninfected and infected females, only uninfected, or only infected females. Fold change values are $\log_2$, and are expressed as virgin minus mated signal, so a negative logFC represents an increase in signal in response to mating while a positive logFC represents a decrease in signal in response to mating. In instances where more than one probe indicated a significant change in expression for a particular gene, the probeset with the largest fold change is listed. GO term enrichment was determined using GOrilla. Upward-pointing arrows indicate genes with increased expression and downward-pointing arrows indicate genes with depressed expression. A Benjamini-Hochberg correction (Benjamini and Hochberg, 1995) was performed to correct for multiple tests, and only GO terms that were significant after controlling for a false discovery rate of 5% were retained.

Eggless females have a transcriptional response to infection that is similar but not identical that of egg-producing females

Our primary question was whether the germline mediates differences between mated and virgin females in their transcriptional response to infection. We found that both virgin and mated eggless females shared increased expression of 117 genes and decreased expression of 18 genes in response to infection (Figure 5.6, Tables B5 and B6). As was the case for females with intact germlines, the genes whose expression increased in response to infection included many known immunity genes such as those encoding antimicrobial peptides (*AttA, AttB, AttC, AttD, CecA1, CecA2, Cec2, CecB, CecC, Def, Dpt, DptB, Dro, Drs, Drs-l*), peptidoglycan recognition proteins (*PGRP-LB, PGRP-LC, PGRP-LF, PGRP-SA, PGRP-SB1, PGRP-SB2, PGRP-SC2, PGRP-SD*) and other known immune system genes (*edin, IM1, IM10, IM18, IM2, IM23, IM3, IM4, spirit, nimB1, Rel, TepII, TsfI, pirk*; Table 5.4, Tables B5 and B6). Thus, the general response to infection is not germline dependent. Notably missing from this list, however, are the *Tot* genes, many of which were upregulated after infection in both virgin and mated egg-producing females. More detailed inspection revealed that *TotA, TotC* and *TotM* are increased in expression after infection in eggless virgins but not in mated eggless females (Figure 5.6). These genes are also included under the three GO terms enriched in eggless virgins: "cellular response to heat," "response to bacterium" and "multi-organism process" (Table 5.4). This suggests that infection-induced changes in the expression of *Turandot* genes may be partly germline dependent, and that differences in *Tot* inducibility between virgin and mated females may be mediated by the germline.

Another notable difference between the infection response of egg-producing compared to eggless females is that eggless females predictably do not show altered expression of genes



Up in response to infection

Gene Symbol	logFC	BH adj. p-value
TotM	-1.80	0.02450
CG30098	-1.29	0.02784
TotA	-1.28	0.04035
TotC	-1.21	0.03834
Gp93	-1.17	0.00003
CG2918	-1.15	0.00360
CG8389	-1.12	0.04863
CG17271	-1.11	0.00001
CG16713	-1.09	0.00303
TRAM	-1.07	0.00006
CG42816	-1.04	0.00022
Ect3	-1.04	0.00564
Idgf3	-1.03	0.00039
Mvl	-1.01	0.00041
CG5493	-1.00	0.01655

Down in response to infeciton

Gene Symbol	logFC	BH adj. p-value
to	2.37	0.00573
CG11854	2.25	0.00265
Try29F	2.09	0.00246
CG15120	1.41	0.00001
Prat2	1.34	0.00098
CG15199	1.25	0.00012
Tret1-2	1.22	0.00320
CG10516	1.16	0.01413
fu12	1.16	0.00002
Men	1.15	0.00235
CG8112	1.13	0.00036
CG1887	1.04	0.02188
Tret1-1	1.04	0.00525
CG42788	1.03	0.00693
CG18673	1.03	0.03084
CG5999	1.01	0.04283

Up in response to infection

Gene Symbol	logFC	BH adj. p-value
TrpA1	-6.47	6.18E-08
CG34296	-1.73	0.00003
Fst	-1.66	0.00283
CG31775	-1.52	0.00030
CG11313	-1.48	0.00005
IM4	-1.27	0.00107
CG30281	-1.21	0.01554
CG12111	-1.21	0.00256
CG6553	-1.16	5.67E-07
CG3505	-1.16	0.00020
Cyp4p3	-1.08	0.00121
NUCB1	-1.08	0.00019
CG16836	-1.07	0.00282
CG18067	-1.05	0.00544
CG13311	-1.05	0.00062
CG13066	-1.04	0.00149
QC	-1.02	0.02355
CG14529	-1.02	0.00004
CG8543	-1.01	0.00239
Cyp309a2	-1.00	0.00026

Down in response to infection

Gene Symbol	logFC	BH adj. p-value
Lsp2	1.34	0.01942
CG4830	1.31	0.01001
Obp83cd	1.12	2.83E-06
UGP	1.10	0.00910
regucalcin	1.04	0.00005

Figure 5.6: The effect of infection on transcript abundance in virgin and mated eggless females. We assayed for genes that exhibited significant 2-fold or greater differences in transcript abundance in virgin, uninfected versus virgin, infected treatments and in mated, uninfected versus mated, infected treatments. We then determined which genes significantly change in transcript abundance due to infection in both virgin and mated females, only virgins, or only mated females. Fold change values are $\log_2$, and are expressed as uninfected minus infected signal, so a negative logFC represents an increase in signal in response to infection while a positive logFC represents a decrease in signal in response to infection. In instances where more than one probe for a particular gene showed significant change in expression, only the probeset with the largest fold change is listed. GO term enrichment was determined using GOrilla and REVIGO was used to reduce lists of GO terms to those least redundant. Upward-pointing arrows indicate genes with increased expression and downward-pointing arrows indicate genes with depressed expression. A Benjamini-Hochberg correction (Benjamini and Hochberg, 1995) was performed to correct for multiple tests, and only GO terms that were significant after controlling for a false discovery rate of 5% were retained.

Table 5.4: Biological process information for genes significantly altered by infection in virgin and/or mated Eggless females.

Gene list	GO term	GO term Description	Corrected p-value	# genes in GO category
Up significantly after infection in BOTH Virgin and Mated females	GO:0006952	defense response	3.97E-39	38
	GO:0042742	defense response to bacterium	3.13E-33	28
	GO:0006955	immune response	8.09E-30	30
	GO:0009607	response to biotic stimulus	1.03E-28	30
	GO:0002376	immune system process	5.84E-28	30
	GO:0006950	response to stress	2.67E-27	45
	GO:0051704	multi-organism process	8.50E-25	31
	GO:0050896	response to stimulus	2.97E-12	51
	GO:0009253	peptidoglycan catabolic process	2.68E-11	8
	GO:0030203	glycosaminoglycan metabolic process	1.09E-08	8
	GO:0016052	carbohydrate catabolic process	2.12E-06	9
	GO:0005976	polysaccharide metabolic process	6.97E-06	11
	GO:0009308	amine metabolic process	5.12E-04	13
	GO:0043900	regulation of multi-organism process	1.54E-03	6
	GO:0008063	Toll signaling pathway	1.59E-03	5
	GO:0035079	polytene chromosome puffing	1.86E-03	3
	GO:0035080	heat shock-mediated polytene chromosome puffing	1.91E-03	3
	GO:0009595	detection of biotic stimulus	1.97E-03	3
	GO:0005975	carbohydrate metabolic process	2.85E-03	13
	GO:0009057	macromolecule catabolic process	5.61E-03	9
	GO:0080134	regulation of response to stress	7.47E-03	6
	GO:0061060	negative regulation of peptidoglycan recognition protein signaling pathway	2.45E-02	2
	GO:0009056	catabolic process	4.90E-02	12
Down significantly after infection in BOTH Virgin and Mated females	No enrichment			
Up significantly after infection in ONLY Virgin females	GO:0034605	cellular response to heat	1.81E-02	3
	GO:0009617	response to bacterium	3.28E-02	4
	GO:0051704	multi-organism process	3.10E-02	5
Down significantly after infection in ONLY Virgin females	No enrichment			
Up significantly after infection in ONLY Mated females	No enrichment			
Down significantly after infection in ONLY Mated females	No enrichment			

encoding vitelline membrane or chorion proteins after infection regardless of mating status (Figure 5.6, Tables B5 and B6). This is not unexpected since the germline-less females do not produce eggs, but it does provide a clear example of a germline-dependent difference in the transcriptional response to infection between virgin and mated females. This is consistent with our model that post-mating suppression of immune defense is related to energetic expenditure on the production of fertile eggs (Short et al., 2012), and a logical extension is that females who produce proportionally more eggs may suffer immunologically to a greater degree.

As we did for egg-producing females, we also assessed whether there were any quantitative differences in the transcriptional response to infection in virgin versus mated eggless females. We identified genes for which the absolute value of (Comparison A – Comparison B) is greater than 1.0, and found only 32 genes that met this basic criterion (Table B10). Of these 32, only six genes showed a nominally significant difference between virgin and mated females (uncorrected p-value < 0.05, Table B10). Only three genes from the list of 35 were also significant in this same comparison in egg-producing females (*takeout*, CG31775, CG32971). *Takeout* (*to*), which is implicated in circadian regulation of feeding behavior (Sarov-Blat et al., 2000), is downregulated in response to infection more strongly in virgins relative to mated females in both egg-producing and eggless females. Feeding behavior has the potential to affect immune defense (Ayres and Schneider, 2009), and it is possible that this result may indicate a germline-independent way in which mating could affect defense. However, the fact that eggless and egg-producing females have so few genes in common for this comparison suggests that most of the differences we observed in egg-producing females (Table 5.2, Table B9) may in some way be contingent on the presence of a germline.

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Conclusions

In 1996, Sheldon and Verhulst presented the simple but revolutionary idea that the ability to fight infection may depend on investment in life-history traits (Sheldon and Verhulst, 1996). They showed that reduced investment in traits like progeny numbers or parental care can directly or indirectly increase investment in immune defense. Their paper is often cited as the intellectual establishment of the field of ecological immunology. Ecological immunology is now more generally defined as the study of the evolution and function of immune defense in the context of an organism's ecology. This includes the effects of biotic factors such as host physiology, sexual selection, life-history trade-offs and host-microbe interactions as well as abiotic factors such as environmental variation. In short, ecological immunology is the study of all the biotic and abiotic factors that determine immune defense regardless of whether they are considered a part of the canonical immune system.

Over the past fifteen years, evidence supporting the notion that ecological factors dramatically affect the evolution and function of immune defense has rapidly accumulated (Martin et al., 2011). However, the mechanisms behind ecological effects on immune defense remain understudied (Schmid-Hempel, 2003). Consider the example of a trade-off between immune defense and reproduction. This might occur because immunity and reproduction share a limited pool of resources. What might this resource be and how is it utilized by each trait? What genes control differential allocation of this resource and how do different alleles result in differential allocation? Answers to these and other mechanistic questions would serve to provide functional and genetic explanations for selective pressures or constraints that may have shaped the evolution of trade-offs.

As ecological immunology has gained momentum, scientists in the field of *Drosophila* immunity have been quickly and elegantly deconstructing the genetic basis of humoral immune system signaling. This work has expanded beyond the humoral immune response and has grown to encompass other arms of the immune system, resulting in an in-depth genetic understanding of much of insect innate immunity (Lemaitre and Hoffmann, 2007). Knowledge of the *Drosophila* innate immune system is by far the most comprehensive of any insect and it is most certainly the best model for genetics of insect immunity. This wealth of functional information has substantially improved our knowledge of insect immune defense, but a full understanding of how insects resist and tolerate infection remains elusive. Ecological immunology data suggest that a more complete understanding of immune defense requires integration of ecological traits.

In *D. melanogaster,* artificial selection for increased immune defense results in decreased egg viability (Ye et al., 2009). This result suggests that the immune system is not genetically isolated from reproductive traits. It has been suggested that immune system activity and reproduction could be antagonistically affected by a shared signaling mechanism (*e.g.* juvenile hormone; Flatt et al., 2005; Rolff and Siva-Jothy, 2002) such that selection for a change in signaling could result in higher immune system activity but concomitantly decreased egg viability. If this is the case, it suggests that limiting the study of immune defense to the signaling mechanisms within the canonical immune system will not be sufficient to understand immune system function.

The work I have done in my dissertation has convinced me that a synthesis of ecological immunology and *Drosophila* immune genetics would be highly beneficial to both fields. Through use of the molecular and genetic tools available in *Drosophila,* ecological immunology would acquire a greatly improved ability to investigate the physiological and genetic

mechanisms underlying ecological effects on immune defense. By viewing immune defense as a trait influenced by myriad factors outside the insect immune system, *Drosophila* immunologists would gain a more comprehensive and ecologically relevant understanding of insect immune defense.

More recent research in *Drosophila* immunology has begun to integrate aspects of ecology into defense, *e.g.* the importance of tolerance (Ayres and Schneider, 2009). These new areas of research are promising and have already improved our understanding of immune defense. My work emphasizes the importance of considering the role of life-history traits in the function and evolution of immune defense and demonstrates the value of investigating the underlying mechanisms of trade-offs in *D. melanogaster.* My dissertation shows that organism-level immune defense is significantly reduced by mating and that this reduction in defense depends on proper formation of the female germline. This suggests that investment in egg production may alter immune defense. I have also shown that antimicrobial peptide gene transcript abundance is lower due to mating over the course of infection and that changes due to infection in the transcript levels of known reproduction and immunity genes vary between virgin and mated females. Taken together, these data suggest important genetic connections between reproduction and immune system signaling.

A crucial next step is to use the power of *Drosophila* as a model genetic system to tease apart the potentially complex genetic and physiological connections between the immune system and life-history traits. It would be valuable to test multiple reproductive mutants to identify specific aspects of reproduction that act to decrease immune system signaling and overall immune defense. For example, female mutants that arrest egg production at varying stages of oogenesis may reveal the specific stages of egg formation that interact with defense. It would

also be informative to assay for reproductive performance in a variety of gain-of-function immunity mutants such as those that cause constitutive activity of Toll and IMD signaling pathways (*e.g.* Libert et al., 2006; Lemaitre et al., 1996). If immune system signaling and reproduction are competing for a shared pool of resources, driving high immune system activity should result in reduced reproductive output. Hypothesized shared signaling mechanisms, such as hormone signaling or insulin signaling could be genetically or chemically altered in a controlled manner to test whether they in fact have antagonistic effects on immunity and reproduction. Large scale mutant screens and association studies, both of which can be conducted in *Drosophila,* would allow for unbiased identification of additional candidate genes likely to be involved in antagonistic pleiotropy. The large numbers of genetic mutants and RNAi lines available in *Drosophila* would allow for careful follow up to determine the nature of the role that these genes may play in the trade-off.

If we hope to understand the overall ability of organisms to resist and tolerate infection, study of *Drosophila* immune defense must continue to proceed beyond the boundaries of the canonical immune system. We must strive to understand the ways in which immune system signaling is affected by genetic and physiological interactions with life-history traits. We also must consider that those life-history traits may affect immune defense independently of the canonical immune system. Comprehension of overall defense will require integration of these interactions into our view of immune defense and into our studies of immune system function.

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APPENDICES

Appendix A: The effect of mating on baseline antimicrobial peptide gene expression.

Multiple studies have demonstrated that mating results in an increase in constitutive transcript abundance of at least one and often many antimicrobial peptide genes, or AMPs (Mcgraw et al., 2004; Lawniczak and Begun, 2004; Peng et al., 2005; Fedorka et al., 2007; Wigby et al., 2008; Innocenti and Morrow, 2009). When I tested the effect of mating on AMP gene expression, however, I found that mated females did not significantly differ from virgins in uninfected AMP gene expression (Chapter 4). I was interested in determining why my results differed from those previously published. I conducted two experiments to address this question. The first investigated whether post-mating changes in constitutive AMP gene expression are genetically variable, and the second investigated the effect of the number of males present during courting and copulation.

Experiment 1:

I first tested for an effect of mating on AMP gene expression across multiple genetic lines. I reasoned that, since post-mating infection resistance varies across strains of flies (Chapter 1), post-mating immune gene expression may vary as well. Historically, I have used the strain Canton S to test post-mating AMP gene expression. It is possible that our failure to detect an increase in AMP expression after mating is specific to Canton S. I wanted to determine whether this is the case or whether my results are generalizable across multiple genetic backgrounds.

Methods: Females were taken from eight strains: Canton S, RAL-362, RAL-639, RAL-375, RAL-315, RAL-437, RAL-786, RAL-486. Males were taken from RAL-358. Canton S is a standard laboratory stock, and the RAL lines are from the *Drosophila* Genetic Reference Panel

(Mackay et al., 2012). All flies were three-day old virgins at the time of mating. Females from each line were mated to single males. Approximately 2.5 hours after mating, mated females were snap frozen in pools of eight. Virgin controls were handled and frozen in parallel with mated females for each genotype. RNA for all samples was extracted using TRIZOL reagent and gene expression of *Defensin, Diptericin A, Drosomycin and Metchnikowen* was measured by qPCR as described in Chapter 4. *Rpl32* was also measured as a reference gene. All samples from a number of lines failed to give a detectable signal for *Defensin* and/or *Diptericin A*. Upon further inspection, I determined that these lines have genetic polymorphisms in the primer sequences of *Defensin* and *Diptericin A* that prevent proper amplification. For these two genes, I treated these samples as missing data. In order to assess whether mating affected AMP expression in mated versus virgin females, I sorted the data by genetic line. Within each genetic line, I tested for an effect of mating status by performing an ANOVA using expression values from all AMP genes as the response variable. I included *Rpl32* values as a continuous variable in the model, replicate experiment as a random effect, and mating treatment and gene as fixed effects. I also tested for an interaction between mating treatment and gene.

Results: I found no effect of mating status on AMP gene expression, suggesting there is no evidence for mating-induced increases in AMP expression in any of the eight lines tested (Table A.1, Figure A.1). I also failed to detect an interaction between mating treatment and gene, suggesting that the lack of mating effect on AMP expression is consistent for all genes measured (Table A.1, Figure A.1). I therefore conclude that our previous result that mating does not alter AMP gene expression is not idiosyncratic. Rather, it appears to be repeatable across multiple genotypes.

Table A.1: Analyses of variance testing the effect of mating status and gene on antimicrobial peptide gene expression in eight genetic lines.

Line	Factor	d.f.	F-value	p-value
Canton S	*Rpl32*	1	8.61	0.0049
	Mating status	1	3.45	0.0689
	Gene	3	40.66	< 0.0001
	Mating status*gene	3	0.63	0.6009
RAL-315	*Rpl32*	1	73.66	<0.0001
	Mating status	1	1.18	0.2942
	Gene	2	9.91	0.0016
	Mating status*gene	2	0.42	0.6649
RAL-362	*Rpl32*	1	74.79	<0.0001
	Mating status	1	0.12	0.7302
	Gene	3	143.10	< 0.0001
	Mating status*gene	3	0.19	0.9046
RAL-375	*Rpl32*	1	9.73	0.0045
	Mating status	1	1.25	0.2742
	Gene	2	86.54	< 0.0001
	Mating status*gene	2	0.81	0.4582
RAL-437	*Rpl32*	1	72.14	<0.0001
	Mating status	1	0.64	0.4307
	Gene	3	108.64	< 0.0001
	Mating status*gene	3	0.35	0.7895
RAL-486	*Rpl32*	1	46.28	<0.0001
	Mating status	1	0.69	0.4123
	Gene	2	38.46	< 0.0001
	Mating status*gene	2	0.13	0.8781
RAL-639	*Rpl32*	1	34.58	<0.0001
	Mating status	1	0.66	0.4304
	Gene	2	130.45	< 0.0001
	Mating status*gene	2	0.61	0.5563
RAL-786	*Rpl32*	1	19.06	0.0005
	Mating status	1	0.00	0.9473
	Gene	1	14.81	0.0014
	Mating status*gene	1	0.25	0.6227

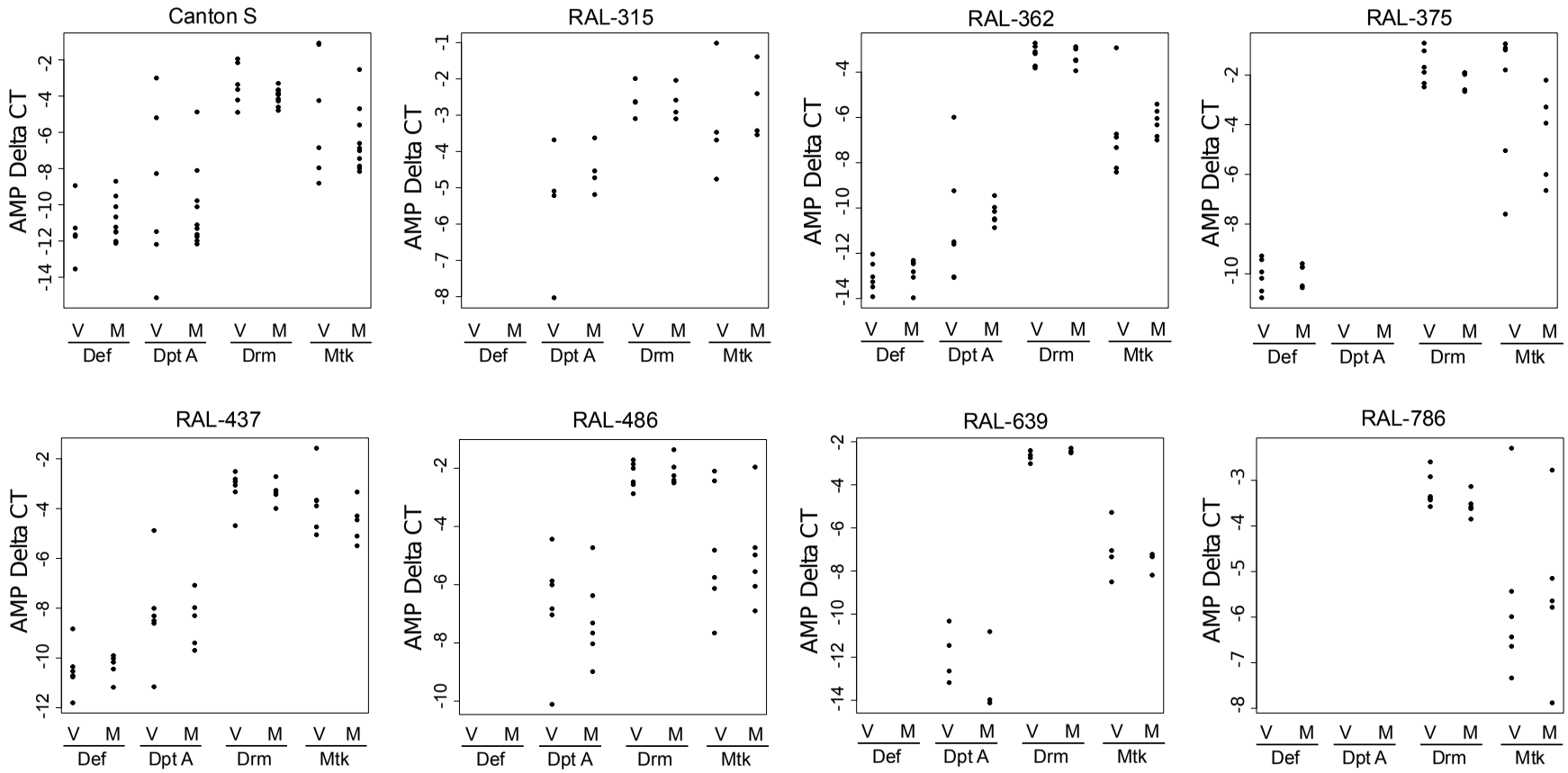

Figure A.1: Log_2 expression values of virgin and mated females for multiple AMP genes in eight inbred lines. Delta critical threshold values ($\Delta CT = CT_{Rpl32}$-CTAMP) are presented for all samples. This corrects for differences in overall mRNA abundance between samples and allows them to be directly compared. Less negative values are indicative of higher AMP transcript abundance relative to *Rpl32* transcript abundance and more negative values are indicative of lower AMP transcript abundance relative to *Rpl32* transcript abundance.

Experiment 2:

I next tested whether the number of males present during courtship and copulation affects female post-mating AMP transcript abundance. In my previous mating experiments, I provided females with a single male, but studies that show increased AMP gene expression due to mating often used two males (Lawniczak and Begun, 2004; Fedorka et al., 2007; Wigby et al., 2008). Males alter transcription of seminal fluid protein genes when they perceive a high risk of male-male competition (Fedorka et al., 2011) and have the ability to alter ejaculate composition based on female mating status (Sirot et al., 2011). Because seminal fluid transfer has been shown to affect baseline AMP expression in females (Mcgraw et al., 2004; Peng et al., 2005), I hypothesized that the perceived mating environment may alter the effect that males have on female baseline AMP expression.

Methods: All flies used were 3-day old virgins from the strain Canton S. I set up experimental matings using single females and either one or two males. Females from both of these mating treatments were allowed to copulate once and then were immediately removed from the presence of males. I also maintained a group of virgin females as controls. Two-three hours after mating, I froze females from each mating treatment in three pools of eight flies and extracted RNA using TRIZOL. I then measured transcript abundance for four AMP genes from all mating treatments using qPCR as described in Chapter 4. I also measured the housekeeping genes *Actin 5C* and *Rpl32* as reference genes. I collected AMP gene expression data over two full replicate experiments (for a total of six pools of eight flies per mating treatment). I assayed for differences in AMP transcript abundance by performing an ANOVA using expression values from all AMP genes as the response variable. I included *Actin 5C* values as a continuous variable in the model and replicate experiment, mating treatment and gene as factors. In addition to

measuring AMP gene expression, during one replicate of the experiment I infected separate females from all mating treatments with *P. rettgeri* at 2-3 hours after mating. Bacterial load of infected females was assayed 24 hours after infection as described in Chapters 2 and 4.

Results: Mated females demonstrated higher bacterial loads than virgins regardless of whether one or two males were present during courting and copulation (Figure A.2). Analysis of variance showed that AMP gene expression was significantly different across mating treatments ($F_{2,63} = 5.08$, $p = 0.009$; Table A.2). A Tukey's test for mating treatment revealed that females exposed to two males demonstrated significantly higher AMP gene expression compared to virgin females but females exposed to single males did not (Table A.3, Figure A.3). However, when *Rpl32* was used as a reference gene, there was no significant effect of mating ($F_{2,63} = 0.94$, $p = 0.39$). The result I obtained when I used *Actin 5C* as a reference suggests that the effect of mating on humoral immune system activity varies depending on whether a second male is present during courtship and copulation. It is possible that males may perceive likely competition from another male and may alter their mating behavior or seminal fluid transfer to reflect this. It is also possible that the female may alter her response to male seminal fluid transfer based on her mating environment. However, my confidence in this result is reduced by the fact that it was not repeatable across multiple reference genes. Further investigation to validate this result is warranted.

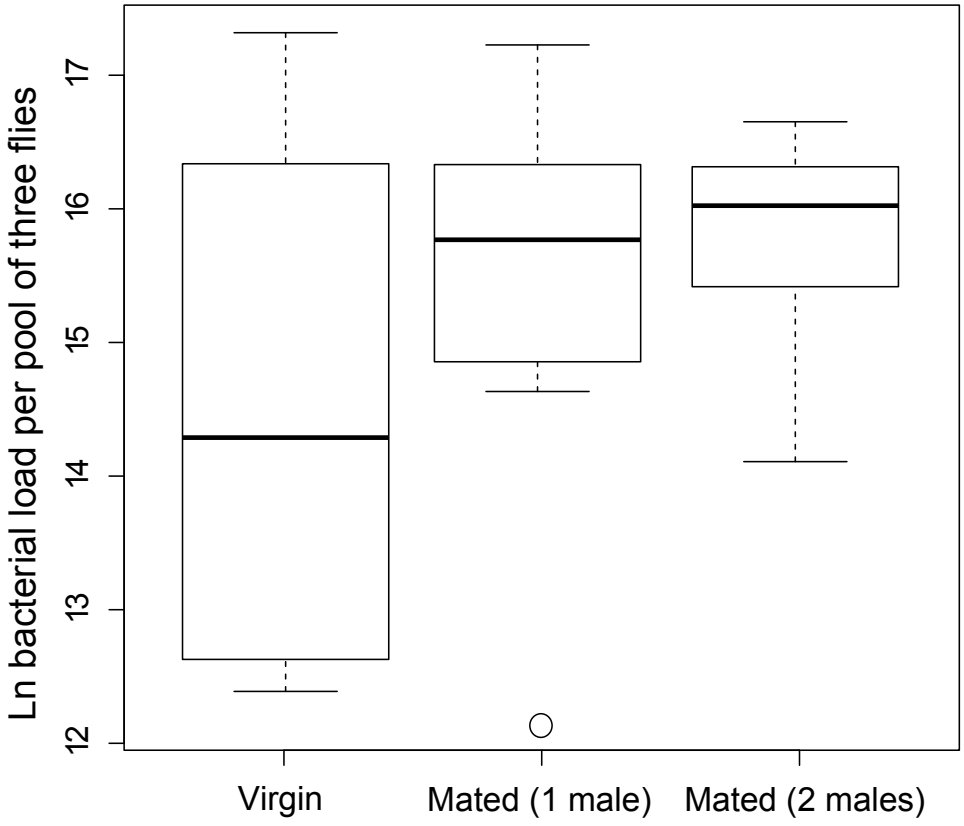

Figure A.2: Bacterial load of females from three mating treatments. Females were either virgin, mated with exposure to a single male or mated with exposure to two males. Mated females were infected 2.5 hours after mating with P. rettgeri bacteria and virgin controls were infected in parallel. Bacterial load was assayed 24 hours post infection.

Table A.2: Analysis of variance testing the effect of mating status and gene on antimicrobial peptide gene expression in virgin females versus mated females exposed to one or two males.

Factor	d.f.	F-value	p-value
Actin 5C	1	4.74	0.0336
Gene	3	48.33	<0.0001
Mating treatment	2	4.85	0.0113
Mating treatment*gene	6	0.57	0.7524

Table A.3: Tukey's test for significant differences in AMP gene expression between specific mating treatments.

Comparison	Estimate	Standard error	p-value
Virgin vs. Mated (1 male)	-0.7368	0.4899	0.2966
Virgin vs. Mated (2 males)	-1.5807	0.5075	0.0080
Mated (1 male) vs. Mated (2 males)	0.8439	0.5009	0.2198

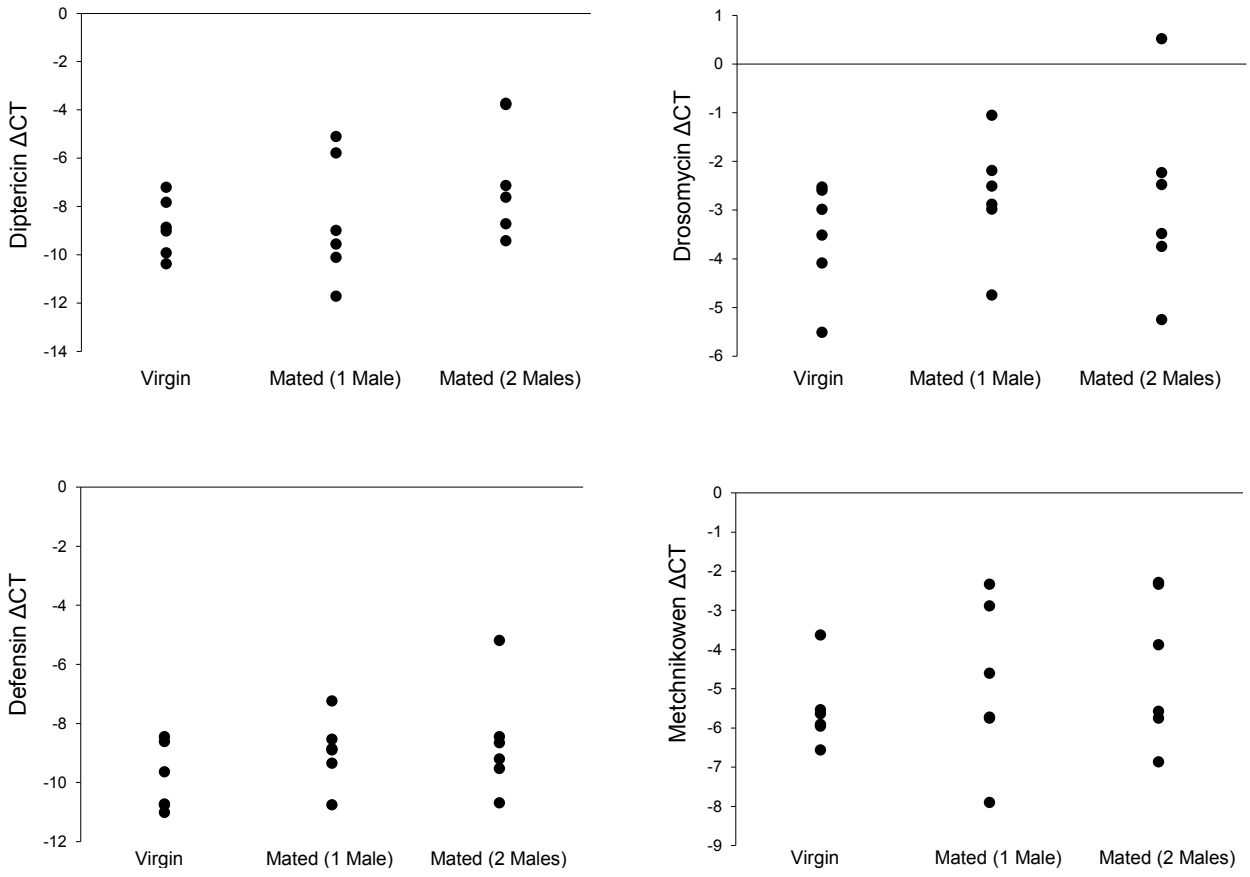

Figure A.3: Log_2 expression values of four AMP genes in females from three mating treatments. Females are either virgin, mated with exposure to a single male or mated with exposure to two males. Delta critical threshold values ($\Delta CT = CT_{Actin5C}\text{-}CT_{AMP}$) are presented for all samples. This corrects for differences in overall mRNA abundance between samples and allows them to be directly compared. Larger values are indicative of higher AMP transcript abundance relative to *Actin5C* and smaller values are indicative of lower AMP transcript abundance relative to *Actin5C*.

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Appendix B.

Supplemental data for Chapter 5

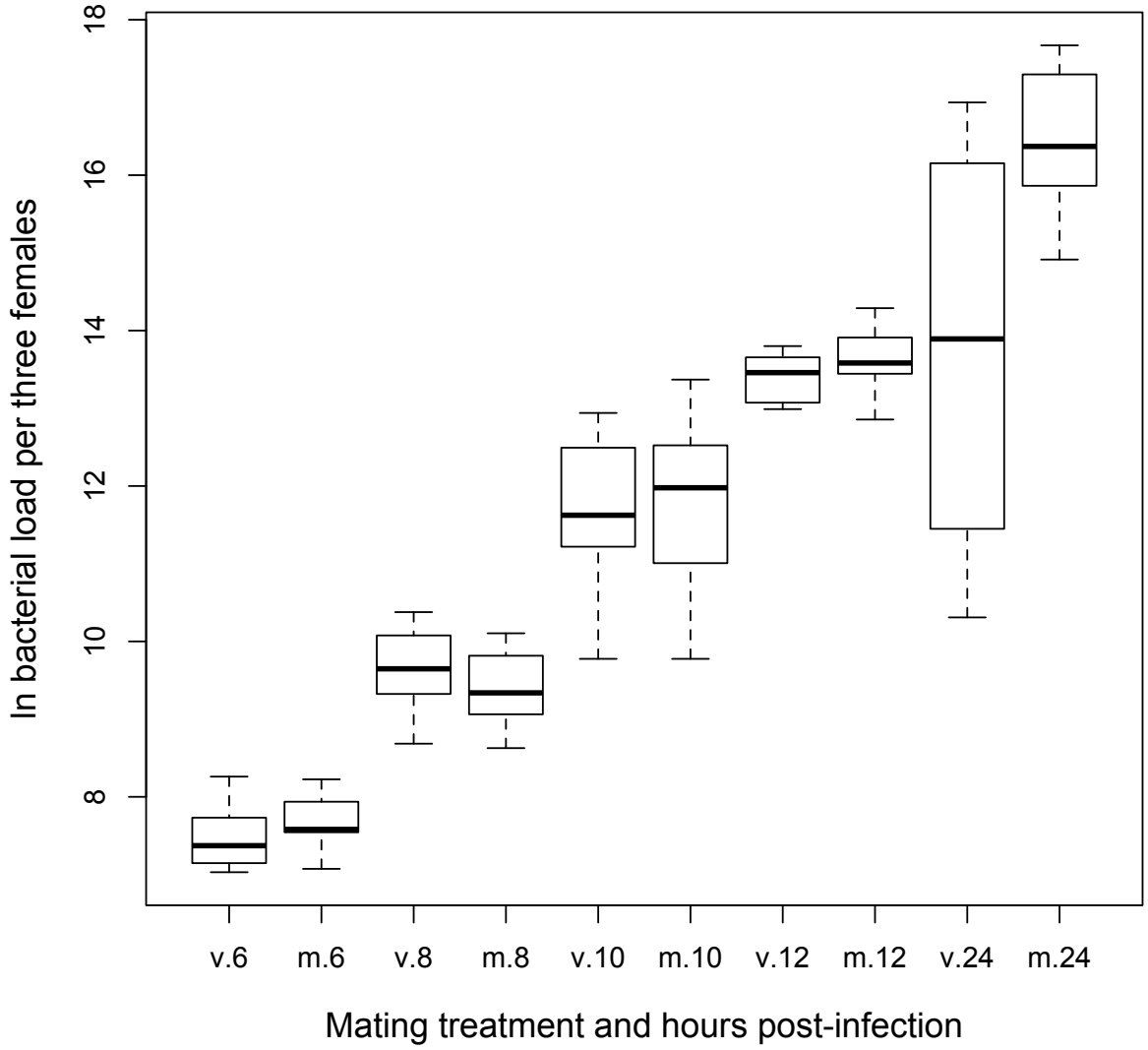

Figure B.1: The effect of mating status on bacterial load at multiple time points post-infection. We infected mated females and virgin controls from the strain Canton S with *P. rettgeri* bacteria at ~2.5 hours post-mating. At six, eight, ten, twelve and twenty-four hours post-infection, we assayed bacterial levels present in virgin and mated females. Females were homogenized in pools of three in sterile LB and an aliquot of homogenate was plated on LB agar plates using a spiral plater (Microbiology International). The number of bacterial colony forming units that grew from that aliquot was used to calculate the number of bacteria per three flies.

Table B1: Log fold change in transcript abundance due to infection in virgin egg-producing females (Comparison A in Figure 5.1). This table contains all probes for genes that were significantly differentially expressed between virgin uninfected egg-producing females and virgin infected egg-producing females.

ProbeUID	Gene name (where available)	logFC (Uninfected virgin - Infected virgin)	p-value	B.H. Adj. p-value	Also significant in mated femles?
20957	CG11501	-4.91	3.45E-04	2.88E-02	NO
4135	CG43085	-2.10	2.08E-05	3.45E-03	NO
15100	Hsp70Bb	-2.02	3.79E-04	3.07E-02	NO
13763	CG13749	-1.48	2.87E-05	4.41E-03	NO
10612	CG15046	-1.47	2.50E-05	3.99E-03	NO
29972	CG43085	-1.46	3.22E-05	4.74E-03	NO
8933	Pu	-1.44	2.53E-06	7.44E-04	NO
4804	CG7367	-1.43	2.32E-04	2.17E-02	NO
4886	CG30088	-1.42	1.39E-05	2.63E-03	NO
9717	CG33459	-1.41	1.19E-06	4.17E-04	NO
16290	CG33468	-1.38	9.67E-07	3.54E-04	NO
22570	Ets21C	-1.33	4.21E-05	5.88E-03	NO
14914	CG8046	-1.33	2.82E-04	2.50E-02	NO
27221	CrebA	-1.33	5.51E-06	1.32E-03	NO
27245	CG7442	-1.30	9.09E-07	3.42E-04	NO
14170	CG14406	-1.30	3.03E-04	2.63E-02	NO
30378	Esyt2	-1.27	3.76E-05	5.36E-03	NO
16567	Cyp6w1	-1.25	7.08E-07	2.96E-04	NO
28194	LpR2	-1.20	5.59E-07	2.50E-04	NO
19395	CG13749	-1.20	1.53E-05	2.81E-03	NO
10368	CG9447	-1.17	3.03E-05	4.61E-03	NO
9786	CG31664	-1.16	9.92E-07	3.59E-04	NO
6344	lectin-24A	-1.13	3.28E-04	2.77E-02	NO
30954	Pif1A	-1.13	4.55E-05	6.22E-03	NO
28121	CG14193	-1.09	1.26E-04	1.38E-02	NO
6236	CG15385	-1.08	1.86E-05	3.20E-03	NO
6596	CG13795	-1.06	3.55E-05	5.12E-03	NO
21775	CG13795	-1.05	4.81E-05	6.45E-03	NO
28015	CG15385	-1.05	8.86E-05	1.04E-02	NO
30873	Ddc	-1.02	5.10E-04	3.86E-02	NO
25317	RhoGAP18B	-1.02	1.25E-04	1.38E-02	NO
24182	CrebA	-1.01	1.38E-04	1.49E-02	NO
17509	Ect3	-1.01	6.07E-05	7.68E-03	NO
25895	CG15120	1.00	4.80E-04	3.71E-02	NO
4005	CG32425	1.01	2.16E-05	3.53E-03	NO
17803	CG6067	1.02	4.42E-05	6.08E-03	NO
30795	UGP	1.03	2.56E-04	2.33E-02	NO
10726	regucalcin	1.03	6.89E-06	1.51E-03	NO
29202	CG32425	1.04	1.58E-05	2.87E-03	NO
23029	CG1887	1.22	5.39E-06	1.31E-03	NO
13492	CG3348	1.24	5.29E-04	3.97E-02	NO
4815	CG3523	1.31	7.73E-06	1.65E-03	NO
5852	Odc1	1.32	3.42E-06	9.41E-04	NO
8032	CG11854	1.35	5.62E-05	7.21E-03	NO
19132	CG4830	1.36	3.65E-04	2.98E-02	NO
17295	CG10621	1.37	4.43E-06	1.17E-03	NO
11878	CG6704	1.40	7.11E-04	4.83E-02	NO
7839	Jon99Ci	1.41	1.23E-04	1.37E-02	NO
17691	Obp99b	1.44	1.21E-04	1.36E-02	NO
16599	CG12398	1.49	1.23E-04	1.37E-02	NO
8521	Vm34Ca	1.50	1.77E-04	1.79E-02	NO
6542	CG43051	1.56	9.44E-07	3.50E-04	NO
20602	Try29F	1.58	7.26E-04	4.91E-02	NO
13587	fit	1.84	1.01E-04	1.16E-02	NO
27279	to	2.09	8.55E-06	1.78E-03	NO
30532	Lsp1beta	2.13	6.44E-06	1.45E-03	NO
7692	Vm26Ab	2.16	5.03E-06	1.25E-03	NO
11455	Vm34Ca	2.17	3.93E-05	5.52E-03	NO
26629	Vml	2.82	1.91E-06	5.92E-04	NO
4113	Vm34Ca	2.98	6.50E-05	8.08E-03	NO
2473	Vm32E	3.78	1.62E-04	1.69E-02	NO
29810	edin	-8.81	1.79E-08	2.31E-05	YES

18652	CecA1	-8.72	8.48E-08	8.04E-05	YES
31959	edin	-8.72	4.54E-08	5.05E-05	YES
16814	CecA2	-8.50	4.70E-07	2.32E-04	YES
12179	CecA2	-8.45	3.11E-07	1.82E-04	YES
4303	CecC	-8.43	1.11E-08	1.78E-05	YES
31592	CecC	-8.00	3.37E-09	1.21E-05	YES
4300	AttA	-7.85	9.14E-07	3.42E-04	YES
11454	AttD	-7.62	1.16E-06	4.10E-04	YES
27879	AttB	-7.49	1.90E-06	5.92E-04	YES
8070	AttA	-7.34	7.69E-07	3.18E-04	YES
31794	AttA	-6.80	2.34E-07	1.51E-04	YES
22744	CG14322	-6.77	8.95E-09	1.75E-05	YES
23177	DptB	-6.21	1.42E-07	1.09E-04	YES
971	AttC	-6.13	4.46E-06	1.17E-03	YES
31396	DptB	-6.06	4.74E-07	2.32E-04	YES
11140	Dpt	-5.63	8.63E-07	3.42E-04	YES
26123	PGRP-SB1	-5.48	1.02E-07	8.84E-05	YES
12774	CecB	-5.38	1.24E-08	1.90E-05	YES
10953	PGRP-SB1	-5.16	6.66E-08	6.71E-05	YES
1240	Mtk	-5.15	1.33E-07	1.07E-04	YES
27837	IM18	-5.10	1.36E-07	1.07E-04	YES
3715	CG10814	-4.82	1.39E-06	4.52E-04	YES
7229	TotM	-4.69	1.07E-05	2.14E-03	YES
1173	CecB	-4.43	1.31E-04	1.42E-02	YES
29065	TotC	-4.38	4.30E-06	1.15E-03	YES
6203	CG2217	-4.32	6.93E-08	6.77E-05	YES
229	PGRP-SB2	-4.09	1.99E-08	2.41E-05	YES
32174	TotA	-4.06	7.09E-06	1.54E-03	YES
20476	PGRP-LB	-4.03	1.57E-09	7.23E-06	YES
5420	CG13905	-3.91	1.00E-07	8.84E-05	YES
13809	Def	-3.79	4.49E-07	2.32E-04	YES
29001	TotM	-3.75	1.85E-05	3.20E-03	YES
16560	pirk	-3.71	9.60E-09	1.75E-05	YES
26645	pirk	-3.69	1.03E-08	1.75E-05	YES
9914	pirk	-3.65	9.27E-09	1.75E-05	YES
27320	CG30098	-3.57	1.70E-08	2.29E-05	YES
6289	CG30098	-3.52	5.24E-08	5.44E-05	YES
12164	CG4269	-3.43	4.01E-06	1.08E-03	YES
3885	Def	-3.41	4.70E-07	2.32E-04	YES
14390	Fst	-3.34	1.46E-05	2.70E-03	YES
19212	CG31775	-3.30	1.10E-05	2.19E-03	YES
14348	CG34054	-3.09	8.93E-09	1.75E-05	YES
31468	CG34054	-3.08	7.95E-09	1.75E-05	YES
13290	Dro	-3.06	1.19E-10	2.79E-06	YES
13593	CG14190	-3.04	1.29E-09	6.91E-06	YES
17438	CG42559	-3.01	1.13E-09	6.91E-06	YES
30684	CecA1	-2.95	1.06E-09	6.91E-06	YES
3201	TepII	-2.91	1.07E-06	3.82E-04	YES
12003	CG13077	-2.78	2.26E-10	2.79E-06	YES
28824	CG13422	-2.76	2.78E-06	8.01E-04	YES
17460	CG30080	-2.72	2.55E-07	1.58E-04	YES
4761	TepII	-2.71	1.99E-07	1.40E-04	YES
7484	CG6361	-2.64	5.96E-09	1.75E-05	YES
1474	CG11425	-2.59	2.00E-07	1.40E-04	YES
21878	CG6188	-2.57	7.39E-06	1.60E-03	YES
18536	PGRP-SC2	-2.55	1.34E-07	1.07E-04	YES
13445	PGRP-SC2	-2.54	7.92E-07	3.23E-04	YES
11763	CG6188	-2.54	8.06E-06	1.71E-03	YES
17355	CG30026	-2.46	3.07E-07	1.82E-04	YES
19926	spirit	-2.45	4.14E-07	2.19E-04	YES
9600	eg	-2.44	5.01E-07	2.41E-04	YES
9501	PGRP-LF	-2.41	1.56E-08	2.24E-05	YES
325	PGRP-LC	-2.32	1.46E-07	1.09E-04	YES
21011	CG43194	-2.26	1.55E-06	4.90E-04	YES
10420	TotA	-2.25	2.38E-07	1.51E-04	YES
19844	TotB	-2.23	5.52E-06	1.32E-03	YES
31207	PGRP-SD	-2.21	4.92E-06	1.25E-03	YES
18732	PGRP-SA	-2.20	4.66E-07	2.32E-04	YES
19061	PGRP-SA	-2.14	5.56E-07	2.50E-04	YES
20699	Drs-l	-2.13	1.55E-06	4.90E-04	YES

22368	TrpA1	-2.11	5.32E-07	2.48E-04	YES
19026	Ect3	-2.10	4.77E-08	5.12E-05	YES
20148	CG14743	-2.05	5.87E-07	2.57E-04	YES
3592	PGRP-LC	-2.03	8.50E-09	1.75E-05	YES
7931	CG11459	-2.03	9.76E-06	2.00E-03	YES
4250	CG42807	-2.02	5.09E-05	6.66E-03	YES
9312	PGRP-LF	-2.02	1.09E-07	9.23E-05	YES
6079	CG9733	-2.01	2.57E-05	4.04E-03	YES
16012	CG18563	-1.98	6.04E-06	1.40E-03	YES
23994	CG6361	-1.91	1.43E-05	2.69E-03	YES
7947	yellow-f	-1.90	3.44E-07	1.98E-04	YES
3152	PGRP-LC	-1.90	3.33E-06	9.34E-04	YES
14378	IM1	-1.89	2.67E-05	4.15E-03	YES
16781	CG43691	-1.88	3.73E-07	2.04E-04	YES
21230	CG8046	-1.86	1.24E-06	4.21E-04	YES
12091	Uro	-1.84	1.65E-04	1.70E-02	YES
3209	CG16712	-1.82	3.51E-08	4.04E-05	YES
17020	Drs	-1.81	2.66E-07	1.61E-04	YES
31068	CG14529	-1.81	1.00E-08	1.75E-05	YES
14034	CG32284	-1.80	5.46E-07	2.50E-04	YES
16565	CG9989	-1.76	1.45E-06	4.67E-04	YES
14849	Pu	-1.73	3.05E-05	4.61E-03	YES
28059	Tsf1	-1.70	2.53E-04	2.32E-02	YES
7970	Mvl	-1.69	2.27E-06	6.85E-04	YES
17975	Spn88Eb	-1.65	9.60E-06	1.98E-03	YES
12288	Rgk1	-1.65	2.29E-07	1.51E-04	YES
10796	CG14529	-1.64	2.40E-07	1.51E-04	YES
30759	Tsf1	-1.61	7.01E-04	4.81E-02	YES
31247	Tsf1	-1.60	5.21E-04	3.93E-02	YES
24839	CG5527	-1.55	2.45E-05	3.95E-03	YES
10439	Idgf3	-1.53	1.26E-05	2.43E-03	YES
9973	CG9989	-1.52	2.23E-05	3.63E-03	YES
21376	Or22c	-1.52	1.37E-06	4.52E-04	YES
27244	IM10	-1.51	1.78E-04	1.79E-02	YES
2530	CG13641	-1.51	1.48E-04	1.57E-02	YES
24057	PGRP-LB	-1.51	5.64E-06	1.33E-03	YES
4927	CG33460	-1.50	1.75E-05	3.07E-03	YES
14019	Rel	-1.50	8.34E-06	1.75E-03	YES
2068	Rel	-1.47	2.54E-05	4.03E-03	YES
17627	CG5849	-1.46	1.55E-05	2.83E-03	YES
22102	Rel	-1.44	6.17E-07	2.62E-04	YES
21357	Cyp4p3	-1.41	5.61E-06	1.33E-03	YES
3720	CG14762	-1.40	9.86E-05	1.14E-02	YES
7859	Cyp4p3	-1.40	6.05E-07	2.60E-04	YES
31071	CG12111	-1.39	1.22E-05	2.37E-03	YES
11104	CG14642	-1.37	1.99E-07	1.40E-04	YES
6549	CG34215	-1.35	8.55E-07	3.42E-04	YES
10547	CG31516	-1.31	4.98E-06	1.25E-03	YES
21656	CG16713	-1.31	1.04E-05	2.12E-03	YES
9698	CG34427	-1.29	1.57E-04	1.65E-02	YES
5995	CG33470	-1.29	6.03E-05	7.68E-03	YES
3861	CG4725	-1.29	6.96E-05	8.46E-03	YES
7933	Idgf3	-1.28	3.15E-06	8.90E-04	YES
12744	CG16965	-1.27	4.67E-06	1.21E-03	YES
16923	Gadd45	-1.24	2.82E-05	4.34E-03	YES
8604	CG5527	-1.24	1.21E-06	4.19E-04	YES
17787	CG16713	-1.23	6.08E-05	7.68E-03	YES
5550	CG15550	-1.21	4.76E-05	6.44E-03	YES
13048	CG34296	-1.20	2.57E-05	4.04E-03	YES
3617	Mec2	-1.19	1.65E-05	2.96E-03	YES
27871	CG3505	-1.16	5.16E-07	2.45E-04	YES
22404	Cec-Psi2	-1.14	1.68E-05	2.98E-03	YES
6455	CG5791	-1.14	1.89E-04	1.87E-02	YES
9756	IM3	-1.13	1.21E-04	1.35E-02	YES
30946	Gadd45	-1.12	3.07E-05	4.61E-03	YES
16291	Idgf3	-1.11	2.97E-06	8.46E-04	YES
14970	CG15023	-1.09	1.06E-05	2.14E-03	YES
16766	Cyp309a2	-1.06	6.22E-06	1.41E-03	YES
24453	nimB1	-1.03	4.81E-04	3.71E-02	YES
29668	Tsf1	-1.03	1.20E-04	1.35E-02	YES

28877	PGRP-LB	-1.03	3.88E-04	3.13E-02	YES
4047	CG14762	-1.01	1.33E-05	2.54E-03	YES
8392	CG34136	1.00	2.01E-04	1.95E-02	YES
16073	CG13998	1.01	6.64E-04	4.61E-02	YES
14385	CG17124	1.10	8.29E-06	1.75E-03	YES
23871	CG30059	1.12	7.24E-05	8.74E-03	YES
7109	lectin-28C	1.16	6.38E-04	4.50E-02	YES
8316	Obp83cd	1.19	4.72E-06	1.22E-03	YES
29719	psd	1.23	2.10E-05	3.47E-03	YES
28440	Acp65Aa	1.24	9.09E-07	3.42E-04	YES
18628	Acp65Aa	1.24	2.44E-06	7.27E-04	YES
7219	CG12057	1.30	2.52E-04	2.32E-02	YES
23486	CG1648	1.31	5.89E-07	2.57E-04	YES
15322	CG16758	1.33	1.45E-05	2.70E-03	YES
6583	Jon99Fi	1.37	1.65E-04	1.70E-02	YES
19603	Prat2	1.56	6.72E-06	1.50E-03	YES
850	CG4950	1.67	1.13E-05	2.22E-03	YES
17378	psd	1.80	1.13E-05	2.22E-03	YES
24855	Vm26Ac	1.81	6.54E-05	8.11E-03	YES
21669	1-Dec	2.12	1.07E-04	1.21E-02	YES
29589	Obp99a	2.23	3.83E-07	2.06E-04	YES
4479	CG9837	2.28	3.59E-07	2.03E-04	YES
20454	Obp99a	2.53	2.16E-07	1.48E-04	YES
30302	Vml	2.74	9.04E-07	3.42E-04	YES
5200	Lsp2	2.87	6.21E-06	1.41E-03	YES
16849	Lsp2	2.92	2.13E-06	6.52E-04	YES
15638	CG8147	3.21	2.13E-05	3.50E-03	YES

Table B2: Log fold change in transcript abundance due to infection in mated egg-producing females (Comparison B in Figure 5.1). This table contains all probes for genes that were significantly differentially expressed between mated uninfected egg-producing females and mated infected egg-producing females.

ProbeUID	Gene name (where available)	logFC (Uninfected mated - Infected mated)	p-value	B.H. Adj. p-value	Also significant in virgins?
3102	CG31775	-4.97	1.45E-07	1.10E-04	NO
22213	CG31775	-3.15	5.94E-10	2.79E-06	NO
6382	CG4757	-2.94	5.96E-04	3.91E-02	NO
30225	IM23	-2.54	2.00E-04	1.76E-02	NO
2374	Ugt37b1	-2.23	3.71E-06	9.14E-04	NO
6913	TotX	-2.11	2.13E-04	1.83E-02	NO
13478	CG9463	-1.97	7.71E-05	8.87E-03	NO
7036	IM4	-1.51	1.92E-05	3.09E-03	NO
27775	CG13641	-1.48	5.71E-04	3.76E-02	NO
14847	yellow-f	-1.47	2.42E-05	3.69E-03	NO
11530	Lip3	-1.45	1.81E-04	1.65E-02	NO
25948	CG32023	-1.41	2.46E-05	3.72E-03	NO
27714	CG15533	-1.41	7.99E-06	1.61E-03	NO
20835	CG15065	-1.38	9.01E-05	9.88E-03	NO
9322	CG9780	-1.37	1.80E-07	1.32E-04	NO
5100	CG34291	-1.33	1.80E-04	1.65E-02	NO
29898	CG6495	-1.33	4.23E-06	9.83E-04	NO
21062	CG34291	-1.32	2.08E-04	1.80E-02	NO
15375	CG13311	-1.31	6.09E-04	3.96E-02	NO
15279	CG9396	-1.29	1.08E-05	1.96E-03	NO
7703	IM2	-1.28	6.33E-04	4.04E-02	NO
31738	IM4	-1.25	5.64E-05	6.91E-03	NO
26467	IM3	-1.23	4.38E-05	5.74E-03	NO
2835	Smvt	-1.23	3.64E-04	2.71E-02	NO
29493	CG16836	-1.22	5.34E-07	2.71E-04	NO
26212	CG6553	-1.19	8.75E-05	9.79E-03	NO
31343	Smvt	-1.18	6.83E-04	4.27E-02	NO
25423	CG16836	-1.18	1.44E-06	4.83E-04	NO
15847	CG34426	-1.15	3.75E-05	5.19E-03	NO
7261	SerT	-1.13	1.55E-06	4.96E-04	NO
5954	CG7017	-1.13	9.22E-07	3.71E-04	NO
14574	CG5791	-1.12	7.02E-05	8.26E-03	NO
31766	CG4725	-1.09	1.20E-06	4.36E-04	NO

2918	CG8550	-1.08	4.29E-05	5.67E-03	NO
10332	Spn4	-1.06	2.85E-06	7.66E-04	NO
18077	CG9649	-1.06	6.02E-05	7.24E-03	NO
6981	hgo	-1.05	2.64E-04	2.12E-02	NO
13163	Spn1	-1.04	9.15E-06	1.76E-03	NO
27476	CG6553	-1.02	4.55E-05	5.87E-03	NO
11732	E5	-1.02	5.99E-05	7.24E-03	NO
6697	CG8449	-1.01	1.53E-04	1.48E-02	NO
22942	hgo	-1.01	8.66E-04	4.98E-02	NO
12156	Spn1	-1.00	3.43E-04	2.61E-02	NO
13860	Spn28D	-1.00	3.14E-04	2.42E-02	NO
6613	ndl	1.01	1.77E-06	5.43E-04	NO
28438	Orct	1.02	1.15E-06	4.26E-04	NO
21752	CG13042	1.02	5.42E-04	3.62E-02	NO
20147	shf	1.05	2.37E-06	6.76E-04	NO
10797	CG9747	1.07	2.80E-05	4.06E-03	NO
4704	CG3999	1.11	2.54E-05	3.78E-03	NO
28101	CG14095	1.11	3.49E-05	4.90E-03	NO
29458	CG34205	1.12	5.09E-05	6.43E-03	NO
22519	CG31778	1.15	2.36E-06	6.76E-04	NO
27369	CG17751	1.16	1.50E-04	1.47E-02	NO
2320	Tret1-2	1.17	1.08E-05	1.96E-03	NO
19158	CG17751	1.20	3.95E-07	2.23E-04	NO
23091	CG31778	1.21	2.52E-06	7.00E-04	NO
2680	GATAd	1.23	6.97E-04	4.31E-02	NO
25497	HLHmgamma	1.23	8.32E-05	9.39E-03	NO
19476	alpha-Est2	1.23	1.37E-06	4.74E-04	NO
2498	amd	1.26	7.55E-04	4.52E-02	NO
21922	CG34278	1.32	3.85E-05	5.25E-03	NO
24620	CG34136	1.33	1.94E-05	3.10E-03	NO
24554	HLHm5	1.38	8.65E-04	4.98E-02	NO
18261	CG34247	1.38	2.13E-06	6.23E-04	NO
3731	Damm	1.43	8.06E-05	9.18E-03	NO
22773	Vha16-2	1.52	1.29E-05	2.29E-03	NO
10474	CG31437	1.52	3.17E-06	8.11E-04	NO
11674	lectin-28C	1.61	4.26E-04	3.03E-02	NO
27011	CG34367	1.63	7.92E-06	1.61E-03	NO
30160	CG17738	1.89	4.14E-06	9.74E-04	NO
17541	Ir7c	2.07	2.08E-07	1.40E-04	NO
29810	edin	-9.01	1.44E-08	2.01E-05	YES
31959	edin	-8.92	3.63E-08	4.04E-05	YES
4303	CecC	-8.61	8.96E-09	1.37E-05	YES
31592	CecC	-8.42	1.99E-09	4.57E-06	YES
18652	CecA1	-8.25	1.47E-07	1.10E-04	YES
16814	CecA2	-7.75	1.13E-06	4.26E-04	YES
12179	CecA2	-7.72	7.37E-07	3.17E-04	YES
22744	CG14322	-7.12	5.46E-09	9.78E-06	YES
4300	AttA	-6.72	3.93E-06	9.44E-04	YES
27879	AttB	-6.41	8.00E-06	1.61E-03	YES
8070	AttA	-6.20	3.77E-06	9.18E-04	YES
11140	Dpt	-5.98	4.85E-07	2.63E-04	YES
12774	CecB	-5.94	4.59E-09	9.51E-06	YES
11454	AttD	-5.86	1.31E-05	2.30E-03	YES
31794	AttA	-5.85	9.93E-07	3.95E-04	YES
27837	IM18	-5.69	4.69E-08	4.87E-05	YES
26123	PGRP-SB1	-5.56	8.70E-08	7.92E-05	YES
19212	CG31775	-5.36	1.13E-07	9.08E-05	YES
23177	DptB	-5.30	6.58E-07	3.01E-04	YES
31396	DptB	-5.20	2.02E-06	6.04E-04	YES
971	AttC	-5.05	2.56E-05	3.79E-03	YES
10953	PGRP-SB1	-4.98	9.53E-08	8.08E-05	YES
3715	CG10814	-4.95	1.09E-06	4.17E-04	YES
1240	Mtk	-4.90	2.17E-07	1.40E-04	YES
6203	CG2217	-4.80	2.46E-08	2.83E-05	YES
20476	PGRP-LB	-4.75	2.93E-10	2.32E-06	YES
229	PGRP-SB2	-4.54	6.93E-09	1.12E-05	YES
1173	CecB	-4.51	1.13E-04	1.17E-02	YES
14348	CG34054	-3.59	1.96E-09	4.57E-06	YES
31468	CG34054	-3.55	1.93E-09	4.57E-06	YES

13593	CG14190	-3.54	2.71E-10	2.32E-06	YES
29065	TotC	-3.51	3.14E-05	4.47E-03	YES
3885	Def	-3.45	4.22E-07	2.34E-04	YES
26645	pirk	-3.44	2.08E-08	2.48E-05	YES
16560	pirk	-3.44	2.04E-08	2.48E-05	YES
9914	pirk	-3.39	1.97E-08	2.48E-05	YES
32174	TotA	-3.36	3.84E-05	5.25E-03	YES
7229	TotM	-3.32	2.01E-04	1.76E-02	YES
31207	PGRP-SD	-3.31	1.02E-07	8.44E-05	YES
5420	CG13905	-3.30	5.24E-07	2.71E-04	YES
17460	CG30080	-3.27	4.18E-08	4.49E-05	YES
28824	CG13422	-3.24	6.12E-07	2.94E-04	YES
13290	Dro	-3.21	7.23E-11	1.50E-06	YES
17438	CG42559	-3.20	6.07E-10	2.79E-06	YES
13809	Def	-3.18	2.39E-06	6.76E-04	YES
12164	CG4269	-3.16	8.49E-06	1.69E-03	YES
30684	CecA1	-3.06	7.22E-10	2.91E-06	YES
9600	eg	-2.99	6.88E-08	6.72E-05	YES
20699	Drs-l	-2.91	7.60E-08	7.20E-05	YES
19926	spirit	-2.87	8.85E-08	7.92E-05	YES
31247	Tsf1	-2.84	3.95E-06	9.44E-04	YES
30759	Tsf1	-2.74	8.82E-06	1.73E-03	YES
29001	TotM	-2.73	2.61E-04	2.10E-02	YES
21878	CG6188	-2.70	4.74E-06	1.06E-03	YES
7484	CG6361	-2.68	5.18E-09	9.78E-06	YES
28059	Tsf1	-2.66	5.35E-06	1.15E-03	YES
12003	CG13077	-2.65	3.61E-10	2.32E-06	YES
9501	PGRP-LF	-2.64	6.24E-09	1.06E-05	YES
11763	CG6188	-2.57	7.14E-06	1.51E-03	YES
325	PGRP-LC	-2.56	5.46E-08	5.50E-05	YES
17355	CG30026	-2.40	3.91E-07	2.23E-04	YES
6289	CG30098	-2.34	2.72E-06	7.36E-04	YES
16012	CG18563	-2.33	1.36E-06	4.74E-04	YES
4761	TepII	-2.31	9.18E-07	3.71E-04	YES
27320	CG30098	-2.31	1.19E-06	4.36E-04	YES
27244	IM10	-2.30	4.79E-06	1.06E-03	YES
9973	CG9989	-2.23	6.38E-07	2.98E-04	YES
4250	CG42807	-2.22	2.24E-05	3.49E-03	YES
3592	PGRP-LC	-2.15	4.72E-09	9.51E-06	YES
19061	PGRP-SA	-2.14	5.52E-07	2.74E-04	YES
18732	PGRP-SA	-2.11	7.19E-07	3.17E-04	YES
16565	CG9989	-2.10	2.70E-07	1.62E-04	YES
14390	Fst	-2.07	7.33E-04	4.44E-02	YES
3152	PGRP-LC	-2.06	1.50E-06	4.94E-04	YES
14378	IM1	-2.03	1.40E-05	2.42E-03	YES
23994	CG6361	-2.01	9.05E-06	1.75E-03	YES
5995	CG33470	-1.94	1.56E-06	4.96E-04	YES
6079	CG9733	-1.92	3.70E-05	5.16E-03	YES
24453	nimB1	-1.92	2.37E-06	6.76E-04	YES
10420	TotA	-1.88	1.28E-06	4.58E-04	YES
24057	PGRP-LB	-1.88	7.08E-07	3.17E-04	YES
18536	PGRP-SC2	-1.88	2.56E-06	7.05E-04	YES
17627	CG5849	-1.84	1.84E-06	5.58E-04	YES
6455	CG5791	-1.82	3.06E-06	8.07E-04	YES
3201	TepII	-1.82	7.49E-05	8.68E-03	YES
31068	CG14529	-1.79	1.11E-08	1.63E-05	YES
22368	TrpA1	-1.78	2.68E-06	7.33E-04	YES
13445	PGRP-SC2	-1.72	2.77E-05	4.04E-03	YES
9312	PGRP-LF	-1.69	6.01E-07	2.93E-04	YES
20148	CG14743	-1.69	3.60E-06	8.93E-04	YES
29668	Tsf1	-1.69	1.44E-06	4.83E-04	YES
16781	CG43691	-1.69	1.03E-06	4.04E-04	YES
12288	Rgk1	-1.68	1.87E-07	1.34E-04	YES
17787	CG16713	-1.67	4.24E-06	9.83E-04	YES
14034	CG32284	-1.66	1.15E-06	4.26E-04	YES
14019	Rel	-1.65	3.51E-06	8.85E-04	YES
17020	Drs	-1.65	6.64E-07	3.01E-04	YES
21011	CG43194	-1.64	2.87E-05	4.13E-03	YES
2530	CG13641	-1.63	7.80E-05	8.94E-03	YES
21656	CG16713	-1.60	1.57E-06	4.96E-04	YES

14970	CG15023	-1.59	2.98E-07	1.75E-04	YES
7947	yellow-f	-1.59	1.91E-06	5.75E-04	YES
22404	Cec-Psi2	-1.58	7.93E-07	3.32E-04	YES
12091	Uro	-1.58	5.31E-04	3.57E-02	YES
7859	Cyp4p3	-1.57	1.94E-07	1.35E-04	YES
8604	CG5527	-1.55	1.44E-07	1.10E-04	YES
6549	CG34215	-1.53	2.62E-07	1.62E-04	YES
19844	TotB	-1.52	1.52E-04	1.48E-02	YES
21376	Or22c	-1.49	1.66E-06	5.19E-04	YES
21230	CG8046	-1.49	1.01E-05	1.90E-03	YES
2068	Rel	-1.48	2.48E-05	3.74E-03	YES
22102	Rel	-1.47	4.89E-07	2.63E-04	YES
24839	CG5527	-1.45	4.47E-05	5.78E-03	YES
16923	Gadd45	-1.44	7.68E-06	1.59E-03	YES
7931	CG11459	-1.41	2.15E-04	1.83E-02	YES
19026	Ect3	-1.39	2.51E-06	7.00E-04	YES
16766	Cyp309a2	-1.38	5.38E-07	2.71E-04	YES
5550	CG15550	-1.37	1.57E-05	2.62E-03	YES
10796	CG14529	-1.36	1.38E-06	4.75E-04	YES
3861	CG4725	-1.36	4.45E-05	5.78E-03	YES
11104	CG14642	-1.36	2.14E-07	1.40E-04	YES
13048	CG34296	-1.33	1.05E-05	1.94E-03	YES
7970	Mvl	-1.33	2.05E-05	3.24E-03	YES
3209	CG16712	-1.33	7.38E-07	3.17E-04	YES
31071	CG12111	-1.29	2.38E-05	3.66E-03	YES
28877	PGRP-LB	-1.29	6.25E-05	7.48E-03	YES
4047	CG14762	-1.27	1.55E-06	4.96E-04	YES
17975	Spn88Eb	-1.27	9.43E-05	1.03E-02	YES
21357	Cyp4p3	-1.27	1.52E-05	2.58E-03	YES
3617	Mec2	-1.26	9.81E-06	1.86E-03	YES
14849	Pu	-1.24	4.53E-04	3.17E-02	YES
9756	IM3	-1.24	5.45E-05	6.71E-03	YES
1474	CG11425	-1.22	1.77E-04	1.64E-02	YES
3720	CG14762	-1.20	3.47E-04	2.63E-02	YES
9698	CG34427	-1.17	3.38E-04	2.57E-02	YES
10439	Idgf3	-1.16	1.38E-04	1.37E-02	YES
30946	Gadd45	-1.15	2.38E-05	3.66E-03	YES
12744	CG16965	-1.14	1.24E-05	2.21E-03	YES
27871	CG3505	-1.10	8.29E-07	3.43E-04	YES
7933	Idgf3	-1.09	1.34E-05	2.35E-03	YES
4927	CG33460	-1.04	3.85E-04	2.80E-02	YES
10547	CG31516	-1.02	4.42E-05	5.77E-03	YES
16291	Idgf3	-1.01	7.45E-06	1.56E-03	YES
16073	CG13998	1.01	6.91E-04	4.30E-02	YES
14385	CG17124	1.09	8.95E-06	1.74E-03	YES
7219	CG12057	1.10	8.69E-04	4.98E-02	YES
29719	psd	1.11	5.36E-05	6.69E-03	YES
18628	Acp65Aa	1.12	5.94E-06	1.27E-03	YES
23486	CG1648	1.15	2.12E-06	6.23E-04	YES
28440	Acp65Aa	1.16	1.67E-06	5.19E-04	YES
17378	psd	1.16	4.34E-04	3.07E-02	YES
6583	Jon99Fi	1.17	5.65E-04	3.73E-02	YES
8316	Obp83cd	1.19	4.79E-06	1.06E-03	YES
15322	CG16758	1.29	1.95E-05	3.10E-03	YES
8392	CG34136	1.39	1.24E-05	2.21E-03	YES
850	CG4950	1.45	3.90E-05	5.26E-03	YES
7109	lectin-28C	1.47	9.98E-05	1.08E-02	YES
19603	Prat2	1.48	1.05E-05	1.94E-03	YES
30302	Vml	1.55	1.48E-04	1.46E-02	YES
23871	CG30059	1.56	3.99E-06	9.45E-04	YES
21669	1-Dec	1.64	7.78E-04	4.62E-02	YES
20454	Obp99a	1.67	1.10E-05	1.99E-03	YES
4479	CG9837	1.74	4.47E-06	1.03E-03	YES
29589	Obp99a	2.08	7.56E-07	3.21E-04	YES
24855	Vm26Ac	2.15	1.48E-05	2.52E-03	YES
16849	Lsp2	2.50	8.88E-06	1.73E-03	YES
5200	Lsp2	2.59	1.57E-05	2.62E-03	YES
15638	CG8147	3.98	3.05E-06	8.07E-04	YES

Table B3: Log fold change in transcript abundance due mating in uninfected egg-producing females (Comparison C in Figure 5.1). This table contains all probes from genes that were significantly differentially expressed between virgin uninfected egg-producing females and mated uninfected egg-producing females.

ProbeUID	Gene name (where available)	logFC (Uninfected virgin - Uninfected mated)	p-value	B.H. Adj. p-value	Also significant in infected females?
22773	Vha16-2	-2.28	2.90E-07	4.63E-05	NO
23225	Sr-CII	-2.06	1.63E-03	1.21E-02	NO
24554	HLHm5	-1.94	5.91E-05	1.13E-03	NO
31189	Ama	-1.86	3.02E-04	3.46E-03	NO
10787	CG34232	-1.81	4.11E-07	5.47E-05	NO
18498	Npc2c	-1.80	1.55E-03	1.16E-02	NO
22879	CG15829	-1.79	4.53E-03	2.64E-02	NO
27494	CG7298	-1.78	2.50E-03	1.66E-02	NO
25497	HLHmgamma	-1.73	4.19E-06	2.01E-04	NO
14108	CG15043	-1.69	5.68E-05	1.10E-03	NO
23833	roX1	-1.68	4.30E-04	4.49E-03	NO
12752	CG34232	-1.66	4.92E-07	5.92E-05	NO
6558	Brd	-1.65	2.46E-04	2.98E-03	NO
21032	roX1	-1.64	7.19E-04	6.53E-03	NO
21922	CG34278	-1.64	5.64E-06	2.43E-04	NO
19628	CG15829	-1.63	7.37E-03	3.78E-02	NO
14390	Fst	-1.60	4.24E-03	2.51E-02	NO
30699	rib	-1.57	2.03E-05	5.50E-04	NO
7931	CG11459	-1.56	9.43E-05	1.54E-03	NO
11417	Tsp29Fa	-1.54	1.20E-05	3.89E-04	NO
30950	CG31253	-1.51	3.48E-03	2.14E-02	NO
2680	GATAd	-1.50	1.46E-04	2.09E-03	NO
22874	bib	-1.50	7.11E-07	7.28E-05	NO
3314	CG5246	-1.46	6.12E-03	3.30E-02	NO
2934	cwo	-1.44	9.53E-05	1.55E-03	NO
6708	sna	-1.43	2.62E-04	3.11E-03	NO
13890	Cyp4ad1	-1.43	2.80E-06	1.60E-04	NO
3262	CG12290	-1.42	4.56E-04	4.68E-03	NO
4375	Fst	-1.42	5.51E-03	3.05E-02	NO
24799	sna	-1.41	6.68E-04	6.19E-03	NO
3669	scb	-1.41	8.25E-04	7.22E-03	NO
3743	CG15044	-1.40	7.89E-07	7.73E-05	NO
15638	CG8147	-1.39	9.74E-03	4.68E-02	NO
17645	Mmp2	-1.38	1.49E-04	2.12E-03	NO
7278	CG9616	-1.38	1.77E-07	3.78E-05	NO
30282	Tsp29Fa	-1.38	5.72E-06	2.46E-04	NO
10343	CG13454	-1.38	8.04E-04	7.09E-03	NO
2498	amd	-1.36	4.26E-04	4.46E-03	NO
13378	Ocho	-1.36	2.02E-03	1.41E-02	NO
27740	HLHmgamma	-1.34	2.97E-05	7.04E-04	NO
1477	CG6870	-1.34	1.14E-03	9.24E-03	NO
18621	Cad88C	-1.32	1.36E-05	4.23E-04	NO
25081	pncr008:3L	-1.31	7.69E-05	1.34E-03	NO
11055	mfas	-1.31	1.37E-07	3.50E-05	NO
26774	CG8852	-1.31	1.08E-04	1.68E-03	NO
3716	SRPK	-1.31	2.70E-03	1.76E-02	NO
29908	pncr008:3L	-1.30	3.81E-05	8.37E-04	NO
26907	CG13936	-1.28	1.33E-06	1.04E-04	NO
1610	CG12825	-1.27	3.74E-04	4.06E-03	NO
28206	Ilp4	-1.27	3.46E-03	2.13E-02	NO
28164	CG42323	-1.25	1.72E-03	1.26E-02	NO
14615	CG10912	-1.25	1.40E-03	1.08E-02	NO
1059	CG15170	-1.25	2.95E-04	3.40E-03	NO
15649	Hr46	-1.25	2.43E-05	6.19E-04	NO
23667	CG7409	-1.25	1.97E-05	5.36E-04	NO
3986	DNApol-gamma35	-1.24	4.79E-04	4.85E-03	NO
11636	CG31269	-1.24	1.25E-05	3.98E-04	NO
18536	PGRP-SC2	-1.24	1.03E-04	1.63E-03	NO
29791	spdo	-1.23	1.28E-06	1.02E-04	NO

10269	Nach	-1.23	2.15E-06	1.40E-04	NO
16626	if	-1.21	3.78E-05	8.33E-04	NO
30398	CG3108	-1.20	2.65E-06	1.54E-04	NO
1132	Tmhs	-1.20	1.60E-06	1.18E-04	NO
18938	tal-AA	-1.19	1.44E-04	2.07E-03	NO
4968	tal-AA	-1.18	1.50E-04	2.13E-03	NO
4555	CG11379	-1.18	1.09E-06	9.40E-05	NO
8668	dpr8	-1.18	5.98E-05	1.14E-03	NO
10407	CG16848	-1.17	2.00E-06	1.35E-04	NO
28438	Orct	-1.17	3.06E-07	4.74E-05	NO
3836	CG7882	-1.17	2.45E-04	2.97E-03	NO
6344	lectin-24A	-1.16	2.76E-04	3.24E-03	NO
4857	Bace	-1.15	7.63E-06	2.94E-04	NO
12360	nrv3	-1.14	6.48E-03	3.45E-02	NO
4109	l(1)sc	-1.14	1.99E-04	2.58E-03	NO
20424	Gef64C	-1.14	6.87E-06	2.75E-04	NO
19253	yip7	-1.13	2.69E-04	3.17E-03	NO
9254	Cyp304a1	-1.13	1.80E-04	2.40E-03	NO
5752	Pbprp2	-1.12	2.10E-04	2.68E-03	NO
13445	PGRP-SC2	-1.12	8.85E-04	7.62E-03	NO
3714	CG5597	-1.12	2.22E-03	1.52E-02	NO
14170	CG14406	-1.12	9.40E-04	7.98E-03	NO
25394	cpo	-1.11	6.44E-04	6.02E-03	NO
19216	CG13203	-1.11	1.51E-05	4.52E-04	NO
5168	CG11320	-1.11	6.95E-05	1.26E-03	NO
20434	Cad88C	-1.09	3.08E-05	7.25E-04	NO
7864	CG4822	-1.08	8.53E-07	7.95E-05	NO
29654	nrv3	-1.08	8.09E-04	7.12E-03	NO
4135	CG43085	-1.08	3.36E-03	2.08E-02	NO
9908	CG13203	-1.08	3.26E-05	7.54E-04	NO
31885	Hr46	-1.08	3.56E-06	1.85E-04	NO
19191	CG13068	-1.07	9.00E-04	7.72E-03	NO
1627	Cpn	-1.07	3.03E-03	1.91E-02	NO
20907	betaTub60D	-1.07	2.81E-08	1.92E-05	NO
2608	Hsromega	-1.06	1.13E-03	9.19E-03	NO
28803	CG5059	-1.06	3.04E-06	1.67E-04	NO
12449	Fas3	-1.06	1.84E-04	2.44E-03	NO
21728	CG2533	-1.06	1.77E-04	2.37E-03	NO
17658	klar	-1.06	1.77E-07	3.78E-05	NO
27689	OstStt3	-1.05	9.08E-03	4.43E-02	NO
23502	CG16848	-1.05	5.03E-06	2.25E-04	NO
9914	pirk	-1.03	8.32E-04	7.26E-03	NO
13798	Ptr	-1.03	6.07E-05	1.15E-03	NO
23580	CG2781	-1.03	1.24E-03	9.84E-03	NO
23309	CG15253	-1.03	3.89E-05	8.48E-04	NO
19821	Ilp4	-1.03	8.46E-03	4.19E-02	NO
15033	Bace	-1.03	5.56E-06	2.40E-04	NO
4624	Sox14	-1.02	9.40E-06	3.38E-04	NO
26645	pirk	-1.02	1.03E-03	8.55E-03	NO
3846	CG8086	-1.02	1.88E-03	1.34E-02	NO
1330	CG6231	-1.02	7.89E-05	1.37E-03	NO
13521	KP78b	-1.02	5.81E-03	3.17E-02	NO
14034	CG32284	-1.02	8.84E-05	1.47E-03	NO
13542	ttk	-1.02	1.25E-06	1.01E-04	NO
6288	scb	-1.02	8.33E-04	7.27E-03	NO
7024	CG43689	-1.02	3.63E-06	1.86E-04	NO
24383	pst	-1.01	4.26E-06	2.03E-04	NO
30956	pst	-1.01	6.07E-04	5.76E-03	NO
3731	Damm	-1.01	1.19E-03	9.53E-03	NO
16560	pirk	-1.00	1.16E-03	9.35E-03	NO
19822	fau	-1.00	4.23E-06	2.02E-04	NO
29499	seq	1.00	3.31E-06	1.77E-04	NO
4590	barr	1.00	7.96E-08	2.94E-05	NO
9419	His4:CG33909	1.00	4.74E-04	4.82E-03	NO
26258	l(3)L1231	1.00	1.12E-04	1.73E-03	NO
3491	pzg	1.00	8.69E-06	3.23E-04	NO
29779	CG11873	1.01	3.01E-03	1.91E-02	NO
16793	Mgat1	1.01	3.01E-06	1.66E-04	NO
32059	vlc	1.01	9.67E-05	1.56E-03	NO
13719	CG10600	1.01	1.14E-03	9.23E-03	NO

9875	CG10984	1.01	1.19E-03	9.53E-03	NO
4449	Cwc25	1.01	1.58E-04	2.20E-03	NO
30916	CycA	1.01	8.57E-05	1.44E-03	NO
7138	DNApol-delta	1.01	7.64E-07	7.62E-05	NO
25641	RPA2	1.01	4.84E-07	5.91E-05	NO
29343	DNApol-alpha50	1.01	3.67E-05	8.18E-04	NO
26449	CG3699	1.02	7.97E-04	7.04E-03	NO
3122	CG1979	1.02	4.89E-04	4.92E-03	NO
8313	BubR1	1.02	2.97E-06	1.64E-04	NO
24151	CG1971	1.02	5.61E-05	1.09E-03	NO
6080	retn	1.02	1.28E-06	1.02E-04	NO
31664	CG8478	1.02	7.96E-08	2.94E-05	NO
21757	Gen	1.03	5.26E-07	6.16E-05	NO
7640	Chrac-14	1.03	3.55E-07	5.09E-05	NO
672	GstD1	1.03	1.09E-03	8.92E-03	NO
8845	mad2	1.04	3.87E-07	5.34E-05	NO
4572	CG6808	1.04	4.67E-07	5.86E-05	NO
3498	CG32479	1.04	6.29E-03	3.37E-02	NO
11192	Kmn1	1.04	2.53E-03	1.68E-02	NO
21885	Nedd4	1.04	2.70E-03	1.76E-02	NO
21824	Acf1	1.04	3.51E-03	2.16E-02	NO
13071	CG14962	1.04	1.48E-05	4.46E-04	NO
4192	CG14478	1.04	2.58E-06	1.53E-04	NO
191	qkr58E-2	1.04	2.25E-03	1.54E-02	NO
3381	mod(mdg4)	1.05	3.19E-04	3.60E-03	NO
13766	Mcm3	1.05	2.30E-06	1.44E-04	NO
6156	kappaB-Ras	1.05	5.12E-06	2.28E-04	NO
6011	CG1024	1.05	4.45E-06	2.08E-04	NO
18401	pr-set7	1.05	8.25E-06	3.12E-04	NO
20917	His2B:CG17949	1.05	5.47E-05	1.07E-03	NO
16326	Mal-A7	1.05	3.31E-07	4.91E-05	NO
11283	CG31195	1.06	1.65E-05	4.79E-04	NO
15358	CG11786	1.06	5.22E-04	5.17E-03	NO
20188	Amyrel	1.07	8.15E-03	4.09E-02	NO
484	png	1.07	2.21E-03	1.52E-02	NO
26117	Chrac-14	1.07	7.78E-07	7.71E-05	NO
13334	Oamb	1.07	3.82E-06	1.90E-04	NO
21220	CG16898	1.07	2.73E-03	1.77E-02	NO
1583	Ephrin	1.07	2.56E-04	3.06E-03	NO
18858	l(2)k05819	1.08	3.23E-05	7.51E-04	NO
9543	Incenp	1.08	1.38E-04	2.01E-03	NO
7985	CG11403	1.08	7.09E-05	1.27E-03	NO
25406	CG8949	1.09	6.70E-06	2.71E-04	NO
15	HmgD	1.09	1.08E-05	3.66E-04	NO
22190	CG42388	1.10	4.73E-04	4.81E-03	NO
2308	CG14237	1.10	4.93E-04	4.95E-03	NO
12660	Dip2	1.10	1.62E-06	1.18E-04	NO
18420	cdc2c	1.10	9.22E-07	8.36E-05	NO
18560	tlk	1.11	2.15E-03	1.49E-02	NO
18702	CG7504	1.11	1.82E-06	1.28E-04	NO
24507	eIF4G2	1.11	4.66E-04	4.76E-03	NO
23611	Skp2	1.11	1.04E-05	3.59E-04	NO
4633	mus301	1.12	2.93E-07	4.63E-05	NO
25287	CG8290	1.12	1.27E-06	1.02E-04	NO
25099	CG8116	1.12	1.95E-03	1.38E-02	NO
14277	QC	1.12	1.03E-06	8.98E-05	NO
31473	CG5379	1.12	6.70E-04	6.20E-03	NO
713	CG13373	1.12	2.09E-03	1.45E-02	NO
31537	CrebB-17A	1.13	6.72E-05	1.23E-03	NO
15677	Src64B	1.13	1.15E-06	9.83E-05	NO
30723	CG42232	1.14	2.79E-08	1.92E-05	NO
17602	chn	1.14	2.92E-05	6.96E-04	NO
27589	CG3107	1.15	5.76E-05	1.11E-03	NO
48	CG32625	1.16	1.68E-03	1.23E-02	NO
15180	esc	1.16	6.87E-05	1.25E-03	NO
19159	dgt4	1.16	3.09E-07	4.74E-05	NO
15755	CG42232	1.17	2.47E-08	1.81E-05	NO
807	CG13689	1.17	1.76E-03	1.28E-02	NO
593	Arc2	1.17	4.87E-04	4.91E-03	NO
27486	Nup153	1.18	2.56E-04	3.06E-03	NO

11609	CG11120	1.18	3.70E-07	5.23E-05	NO
22632	CG9007	1.18	2.51E-06	1.51E-04	NO
10321	CG15237	1.19	5.12E-03	2.90E-02	NO
18693	wisp	1.20	1.26E-06	1.01E-04	NO
7596	CG4068	1.22	4.58E-06	2.12E-04	NO
3762	CG10948	1.23	5.40E-03	3.00E-02	NO
7368	HP5	1.26	9.90E-04	8.31E-03	NO
1423	Oamb	1.26	3.42E-03	2.11E-02	NO
18352	His1:CG33807	1.30	3.08E-06	1.68E-04	NO
28982	BicC	1.32	8.81E-05	1.47E-03	NO
17691	Obp99b	1.33	2.38E-04	2.92E-03	NO
28996	His2B:CG33872	1.34	1.31E-04	1.93E-03	NO
27054	dap	1.34	4.35E-05	9.17E-04	NO
3324	Sirt2	1.36	7.90E-03	3.98E-02	NO
27378	His1:CG33801	1.37	2.36E-06	1.46E-04	NO
23697	Ulp1	1.39	9.35E-06	3.37E-04	NO
19378	Cenp-C	1.39	2.29E-05	5.96E-04	NO
27661	Send1	1.43	1.57E-03	1.17E-02	NO
23963	CG11120	1.43	1.91E-06	1.31E-04	NO
29533	His1:CG31617	1.44	6.62E-08	2.94E-05	NO
233	GlcAT-P	1.47	6.54E-03	3.47E-02	NO
22262	snRNA:U1:21D	1.47	4.67E-05	9.58E-04	NO
296	CG7692	1.47	3.64E-04	3.98E-03	NO
20583	glu	1.50	4.49E-04	4.64E-03	NO
28724	His1:CG33810	1.53	1.41E-08	1.46E-05	NO
514	CG33096	1.62	4.79E-03	2.76E-02	NO
1708	CG8478	1.70	1.48E-03	1.12E-02	NO
17567	Tom	-5.15	2.32E-05	6.02E-04	YES
24855	Vm26Ac	-5.11	3.54E-09	1.04E-05	YES
9916	CG4440	-5.03	2.71E-05	6.65E-04	YES
30302	Vml	-5.01	2.36E-09	9.50E-06	YES
6430	CG10035	-4.93	1.17E-04	1.78E-03	YES
2473	Vm32E	-4.75	2.34E-05	6.03E-04	YES
30160	CG17738	-4.54	7.76E-10	5.00E-06	YES
21669	dec-1	-4.25	1.88E-07	3.93E-05	YES
20206	CG32751	-4.21	2.55E-11	6.03E-07	YES
9509	Jon25Bi	-4.19	6.61E-07	6.93E-05	YES
31495	CG13427	-4.19	5.13E-05	1.03E-03	YES
29032	CG17192	-4.13	5.77E-11	6.20E-07	YES
10448	BobA	-3.98	2.34E-05	6.03E-04	YES
17378	psd	-3.79	8.80E-09	1.42E-05	YES
2855	CG13427	-3.72	2.67E-04	3.15E-03	YES
11455	Vm34Ca	-3.54	4.46E-07	5.74E-05	YES
17541	Ir7c	-3.52	1.04E-09	5.60E-06	YES
2304	CG17192	-3.51	4.08E-07	5.45E-05	YES
18569	BobA	-3.49	2.92E-05	6.96E-04	YES
8521	Vm34Ca	-3.39	1.07E-07	3.26E-05	YES
29719	psd	-3.22	2.07E-09	9.50E-06	YES
9676	CG13465	-3.18	6.04E-05	1.14E-03	YES
10286	Cht9	-3.18	1.21E-08	1.44E-05	YES
4532	sala	-3.08	2.73E-04	3.21E-03	YES
8288	SNCF	-3.03	6.87E-04	6.30E-03	YES
11878	CG6704	-3.01	9.60E-07	8.57E-05	YES
16599	CG12398	-3.01	2.11E-07	4.13E-05	YES
19449	halo	-2.99	1.08E-03	8.89E-03	YES
17996	CG11912	-2.99	7.68E-08	2.94E-05	YES
6613	ndl	-2.97	3.74E-11	6.03E-07	YES
3366	Vm26Aa	-2.96	1.13E-04	1.74E-03	YES
7219	CG12057	-2.92	1.74E-07	3.78E-05	YES
18635	m4	-2.89	2.62E-06	1.53E-04	YES
4113	Vm34Ca	-2.87	9.11E-05	1.50E-03	YES
19132	CG4830	-2.82	6.12E-07	6.56E-05	YES
27011	CG34367	-2.73	5.60E-08	2.82E-05	YES
8739	CG8960	-2.72	6.05E-05	1.14E-03	YES
7692	Vm26Ab	-2.72	5.93E-07	6.43E-05	YES
8243	CG13333	-2.68	5.68E-04	5.50E-03	YES
14556	Pcp	-2.67	1.56E-07	3.73E-05	YES
26487	Fcp3C	-2.66	3.21E-03	2.00E-02	YES
16274	CG34224	-2.63	2.91E-04	3.37E-03	YES

27625	CG8960	-2.58	8.05E-05	1.39E-03	YES
28088	Fcp3C	-2.58	5.30E-03	2.96E-02	YES
18261	CG34247	-2.55	5.48E-09	1.20E-05	YES
12326	CG14915	-2.48	4.75E-03	2.74E-02	YES
10474	CG31437	-2.46	3.08E-08	2.02E-05	YES
25042	Bro	-2.45	3.42E-05	7.77E-04	YES
22903	CG13427	-2.45	8.01E-04	7.06E-03	YES
28101	CG14095	-2.44	2.11E-08	1.67E-05	YES
7149	CG14187	-2.40	3.90E-03	2.35E-02	YES
12091	Uro	-2.40	1.70E-05	4.89E-04	YES
16597	vanin-like	-2.39	4.63E-05	9.52E-04	YES
26563	Jon66Ci	-2.38	5.30E-06	2.33E-04	YES
8496	CG31259	-2.37	6.14E-05	1.15E-03	YES
27036	CG31041	-2.36	1.45E-05	4.43E-04	YES
19951	Bsg25A	-2.33	1.11E-03	9.05E-03	YES
23119	CG13813	-2.32	1.63E-05	4.77E-04	YES
6583	Jon99Fi	-2.29	1.77E-06	1.26E-04	YES
11013	CG14377	-2.28	8.29E-08	2.97E-05	YES
27928	CG31041	-2.28	2.04E-05	5.50E-04	YES
29458	CG34205	-2.26	7.08E-08	2.94E-05	YES
21893	CG15634	-2.25	1.36E-03	1.06E-02	YES
15107	CG10592	-2.23	9.93E-09	1.42E-05	YES
21752	CG13042	-2.21	6.65E-07	6.95E-05	YES
28439	CG11911	-2.21	9.77E-08	3.12E-05	YES
17669	CG13813	-2.19	7.77E-06	2.98E-04	YES
28914	CG34137	-2.14	8.11E-04	7.13E-03	YES
27466	CG33109	-2.12	1.39E-08	1.46E-05	YES
20332	CG18180	-2.11	6.65E-06	2.70E-04	YES
16073	CG13998	-2.11	1.21E-06	9.99E-05	YES
8011	CG33109	-2.10	5.54E-08	2.82E-05	YES
16375	CG10182	-2.09	3.83E-04	4.13E-03	YES
12859	Z600	-2.06	7.99E-03	4.02E-02	YES
9109	Jon65Aii	-2.03	1.89E-07	3.93E-05	YES
13587	fit	-2.02	4.58E-05	9.50E-04	YES
914	nullo	-2.02	3.15E-07	4.79E-05	YES
24559	CG10407	-2.00	2.14E-04	2.70E-03	YES
371	CG14317	-1.96	3.20E-03	2.00E-02	YES
11918	Rtnl1	-1.93	1.17E-08	1.44E-05	YES
22610	CG8303	-1.93	1.28E-08	1.44E-05	YES
31671	CG5550	-1.92	1.39E-04	2.02E-03	YES
5848	CG16959	-1.90	1.83E-07	3.87E-05	YES
16457	wbl	-1.90	3.17E-06	1.71E-04	YES
19743	CG5150	-1.89	7.85E-07	7.71E-05	YES
25010	CG10725	-1.88	1.62E-07	3.73E-05	YES
14899	CG15876	-1.87	2.51E-05	6.29E-04	YES
18266	CG7631	-1.86	7.91E-08	2.94E-05	YES
19212	CG31775	-1.86	1.17E-03	9.41E-03	YES
6055	Jon66Ci	-1.86	2.87E-07	4.62E-05	YES
13937	CG7631	-1.85	6.89E-08	2.94E-05	YES
18324	CG11741	-1.85	6.08E-03	3.29E-02	YES
2530	CG13641	-1.85	2.72E-05	6.67E-04	YES
27584	CG31259	-1.84	1.12E-04	1.72E-03	YES
5928	Ilp4	-1.83	5.09E-04	5.07E-03	YES
3447	CG3344	-1.82	5.05E-06	2.26E-04	YES
13157	CG31869	-1.82	2.25E-07	4.14E-05	YES
23210	gammaTry	-1.81	2.51E-07	4.39E-05	YES
14746	sty	-1.81	3.92E-06	1.93E-04	YES
13908	Pebp1	-1.81	3.88E-05	8.47E-04	YES
24758	Pebp1	-1.79	5.07E-05	1.02E-03	YES
5951	CG9825	-1.78	3.68E-05	8.19E-04	YES
24430	Ela	-1.78	4.60E-08	2.60E-05	YES
21883	CG18265	-1.78	2.36E-05	6.05E-04	YES
3141	CG4734	-1.78	2.26E-05	5.91E-04	YES
9669	Hr46	-1.78	1.30E-08	1.44E-05	YES
12009	Ela	-1.76	1.23E-07	3.46E-05	YES
2654	CG16743	-1.76	2.38E-07	4.28E-05	YES
31223	Cpr65Ax1	-1.75	3.73E-04	4.05E-03	YES
12234	CG10725	-1.74	1.76E-06	1.25E-04	YES
9990	deltaTry	-1.74	3.30E-07	4.91E-05	YES
9690	CG14014	-1.73	1.28E-03	1.01E-02	YES

14829	CG14191	-1.70	1.34E-05	4.21E-04	YES
4917	CG13215	-1.70	1.57E-03	1.18E-02	YES
10797	CG9747	-1.70	4.01E-07	5.45E-05	YES
24214	epsilonTry	-1.68	2.88E-06	1.61E-04	YES
11617	epsilonTry	-1.67	1.91E-06	1.31E-04	YES
22024	CG15531	-1.67	2.41E-07	4.30E-05	YES
10666	m1	-1.66	2.51E-06	1.51E-04	YES
24018	CG13051	-1.66	4.02E-06	1.96E-04	YES
23563	CG7675	-1.66	3.35E-06	1.78E-04	YES
3884	CG12420	-1.64	5.35E-04	5.27E-03	YES
26709	CG7912	-1.63	1.05E-04	1.65E-03	YES
12226	CG4734	-1.62	2.81E-06	1.60E-04	YES
4008	Npc2e	-1.60	9.56E-06	3.40E-04	YES
21042	CG33337	-1.60	9.32E-05	1.53E-03	YES
1378	yellow-k	-1.60	1.46E-04	2.09E-03	YES
3180	Jon65Aiv	-1.60	3.19E-05	7.44E-04	YES
28987	CG18258	-1.60	1.16E-07	3.32E-05	YES
26792	CG14500	-1.59	2.65E-05	6.55E-04	YES
30272	CG18258	-1.59	5.75E-07	6.41E-05	YES
12164	CG4269	-1.58	2.14E-03	1.48E-02	YES
28142	CG4563	-1.57	1.54E-05	4.58E-04	YES
30074	CG10910	-1.56	6.07E-04	5.76E-03	YES
27775	CG13641	-1.55	4.03E-04	4.27E-03	YES
5977	CG7912	-1.55	2.25E-04	2.80E-03	YES
13826	CG34214	-1.55	4.63E-03	2.68E-02	YES
4232	PH4alphaEFB	-1.55	7.24E-08	2.94E-05	YES
24069	CG13063	-1.55	1.43E-05	4.39E-04	YES
28285	CG8785	-1.55	8.47E-06	3.17E-04	YES
2744	CG17571	-1.54	3.39E-05	7.73E-04	YES
14424	PGRP-SC1b	-1.54	1.13E-03	9.18E-03	YES
7900	Jon44E	-1.54	4.04E-05	8.76E-04	YES
21463	CG31324	-1.52	1.77E-04	2.37E-03	YES
24974	CrebA	-1.52	2.01E-07	4.05E-05	YES
12078	thetaTry	-1.52	2.47E-05	6.25E-04	YES
20426	CG4020	-1.51	5.23E-09	1.20E-05	YES
12099	tj	-1.50	5.37E-09	1.20E-05	YES
3727	scw	-1.49	9.73E-03	4.68E-02	YES
14469	CG8773	-1.48	2.60E-06	1.53E-04	YES
19991	CG15673	-1.48	5.84E-06	2.48E-04	YES
3730	CG8628	-1.47	9.75E-05	1.57E-03	YES
3380	Jon74E	-1.47	6.37E-05	1.18E-03	YES
11121	CG15828	-1.47	7.84E-08	2.94E-05	YES
8558	CG9465	-1.47	1.31E-04	1.93E-03	YES
10623	CG3868	-1.47	6.38E-08	2.94E-05	YES
16149	Atet	-1.46	3.94E-08	2.36E-05	YES
5218	CG14624	-1.45	1.07E-05	3.64E-04	YES
28300	CG8952	-1.45	6.71E-08	2.94E-05	YES
1420	CG14120	-1.44	5.38E-06	2.35E-04	YES
20818	CG6885	-1.44	1.24E-03	9.83E-03	YES
2525	CG5326	-1.44	2.70E-03	1.76E-02	YES
6215	CG8952	-1.44	2.24E-07	4.14E-05	YES
29670	CG8834	-1.44	1.09E-04	1.69E-03	YES
31343	Smvt	-1.43	1.57E-04	2.19E-03	YES
3720	CG14762	-1.43	8.61E-05	1.45E-03	YES
15335	bond	-1.42	4.94E-07	5.92E-05	YES
20155	CG15673	-1.42	5.42E-04	5.32E-03	YES
18016	CG31269	-1.41	7.79E-05	1.36E-03	YES
28743	CG17560	-1.39	1.39E-04	2.02E-03	YES
16124	CG17032	-1.39	1.06E-06	9.21E-05	YES
14288	CG31086	-1.39	1.53E-04	2.15E-03	YES
4676	CG5767	-1.38	4.20E-05	8.97E-04	YES
10519	Cad99C	-1.38	1.62E-07	3.73E-05	YES
25036	CG13215	-1.38	8.11E-04	7.13E-03	YES
6126	Peritrophin-15a	-1.38	6.82E-05	1.24E-03	YES
27648	Obp83ef	-1.37	1.68E-07	3.78E-05	YES
14106	CG42397	-1.37	8.45E-05	1.43E-03	YES
16800	CG14427	-1.37	1.87E-03	1.34E-02	YES
17682	CG15254	-1.36	2.86E-06	1.61E-04	YES
5380	CG3819	-1.36	1.48E-05	4.47E-04	YES
5247	CG6839	-1.36	3.25E-06	1.75E-04	YES

11979	CG15255	-1.35	5.17E-06	2.29E-04	YES
27187	tal-AA	-1.35	2.53E-07	4.39E-05	YES
2920	CG31288	-1.34	4.09E-04	4.33E-03	YES
24951	CG7025	-1.33	8.81E-06	3.26E-04	YES
9452	Nrx-1	-1.33	4.61E-04	4.72E-03	YES
16691	CG10469	-1.32	5.12E-06	2.28E-04	YES
24424	CG13992	-1.32	4.24E-06	2.02E-04	YES
28517	Npc2d	-1.32	3.27E-06	1.75E-04	YES
9817	CG12374	-1.32	2.55E-06	1.52E-04	YES
11446	CG4020	-1.32	4.47E-05	9.36E-04	YES
25550	CG7300	-1.32	4.25E-07	5.61E-05	YES
12983	CG30272	-1.32	9.71E-04	8.18E-03	YES
7558	br	-1.31	3.23E-05	7.52E-04	YES
4976	Listericin	-1.31	3.57E-06	1.85E-04	YES
13972	daw	-1.31	9.36E-07	8.42E-05	YES
21510	spo	-1.31	3.85E-05	8.44E-04	YES
18829	Jon25Bii	-1.31	1.63E-06	1.18E-04	YES
17505	CR43264	-1.30	4.70E-04	4.78E-03	YES
22199	zetaTry	-1.30	8.32E-05	1.42E-03	YES
24212	Npc2d	-1.30	1.06E-06	9.20E-05	YES
15930	CG8560	-1.30	5.47E-06	2.38E-04	YES
29303	CrebA	-1.30	1.07E-07	3.26E-05	YES
28544	CG14949	-1.30	5.81E-08	2.84E-05	YES
1461	CG10469	-1.29	7.94E-05	1.37E-03	YES
3212	CG6733	-1.28	5.95E-07	6.43E-05	YES
7862	CG5853	-1.28	4.38E-06	2.06E-04	YES
15652	CG5618	-1.27	4.88E-06	2.22E-04	YES
13676	TpnC47D	-1.27	4.06E-07	5.45E-05	YES
6586	CG3759	-1.27	3.42E-07	5.01E-05	YES
16786	zetaTry	-1.26	6.52E-05	1.20E-03	YES
10701	CG10096	-1.26	2.80E-06	1.60E-04	YES
11177	CG13078	-1.26	1.58E-05	4.67E-04	YES
23590	CG15282	-1.25	5.18E-03	2.92E-02	YES
15410	CG3290	-1.25	1.45E-04	2.08E-03	YES
2079	CG15255	-1.25	1.50E-05	4.50E-04	YES
14062	CG9468	-1.23	1.99E-05	5.40E-04	YES
20281	CG3168	-1.23	1.67E-04	2.28E-03	YES
24618	CG6295	-1.23	7.08E-06	2.80E-04	YES
31666	CG4835	-1.23	3.06E-07	4.74E-05	YES
30393	Jon99Cii	-1.22	2.89E-04	3.36E-03	YES
2835	Smvt	-1.22	3.83E-04	4.13E-03	YES
13370	CG13623	-1.22	1.24E-05	3.96E-04	YES
4861	ftz	-1.21	5.31E-03	2.96E-02	YES
19304	Tig	-1.21	1.33E-07	3.50E-05	YES
32011	Jon25Biii	-1.21	4.68E-06	2.14E-04	YES
3868	CG4653	-1.21	4.53E-05	9.44E-04	YES
15196	CG17475	-1.21	1.62E-06	1.18E-04	YES
17614	CG31267	-1.21	1.28E-07	3.46E-05	YES
30887	TpnC47D	-1.21	4.28E-07	5.61E-05	YES
23621	CG13323	-1.21	4.60E-05	9.50E-04	YES
7573	CR40597	-1.21	4.66E-06	2.14E-04	YES
12561	CG33120	-1.21	8.03E-08	2.94E-05	YES
11209	eloF	-1.20	3.53E-07	5.09E-05	YES
21135	CG8774	-1.19	2.52E-05	6.31E-04	YES
15385	CG6129	-1.19	1.22E-06	1.00E-04	YES
12948	CG15254	-1.19	2.07E-05	5.56E-04	YES
2063	sug	-1.19	4.21E-04	4.42E-03	YES
5811	CG6660	-1.18	3.17E-07	4.80E-05	YES
4479	CG9837	-1.17	1.44E-04	2.07E-03	YES
22115	Orct	-1.17	1.58E-06	1.18E-04	YES
14909	CG6432	-1.17	3.11E-04	3.53E-03	YES
18252	Ag5r2	-1.17	7.82E-05	1.36E-03	YES
15618	kar	-1.16	3.59E-07	5.11E-05	YES
12399	Amy-p	-1.16	1.87E-04	2.47E-03	YES
8235	fon	-1.16	1.04E-05	3.59E-04	YES
14971	LanA	-1.16	7.61E-08	2.94E-05	YES
26045	CG7443	-1.16	9.80E-05	1.57E-03	YES
9423	CG7542	-1.15	2.53E-06	1.52E-04	YES
10977	CG5687	-1.15	2.85E-07	4.62E-05	YES
4662	CG31266	-1.15	4.51E-05	9.41E-04	YES

17977	CG9897	-1.14	1.50E-07	3.68E-05	YES
8021	Rcd2	-1.14	1.61E-06	1.18E-04	YES
17776	CG1461	-1.14	1.16E-07	3.32E-05	YES
17024	Ag5r	-1.14	3.71E-06	1.88E-04	YES
26783	CG42323	-1.14	9.52E-04	8.06E-03	YES
17853	cpo	-1.13	2.23E-07	4.14E-05	YES
5259	Jon99Fii	-1.13	5.22E-04	5.17E-03	YES
21426	veil	-1.13	5.07E-07	6.01E-05	YES
17196	CG6660	-1.13	1.19E-06	9.93E-05	YES
3989	spo	-1.12	1.40E-03	1.08E-02	YES
13492	CG3348	-1.12	1.11E-03	9.03E-03	YES
6493	Yp2	-1.12	5.80E-06	2.48E-04	YES
7667	Obp56a	-1.12	7.17E-04	6.51E-03	YES
7747	CG31233	-1.12	1.58E-04	2.20E-03	YES
31845	CG17571	-1.12	4.19E-06	2.01E-04	YES
10347	LysS	-1.12	6.86E-03	3.59E-02	YES
6151	CG17562	-1.12	9.71E-04	8.18E-03	YES
13778	CG33306	-1.12	5.02E-05	1.01E-03	YES
897	CG7025	-1.12	3.30E-04	3.70E-03	YES
4829	CG31974	-1.12	4.23E-05	8.99E-04	YES
17246	CG7381	-1.12	3.20E-05	7.46E-04	YES
27646	Amy-d	-1.11	1.09E-04	1.69E-03	YES
21190	CG34266	-1.11	3.60E-03	2.20E-02	YES
19523	CG6738	-1.11	2.68E-05	6.58E-04	YES
24795	CG13078	-1.11	2.59E-04	3.09E-03	YES
17236	CG31343	-1.11	4.64E-06	2.13E-04	YES
17924	CG17374	-1.11	6.95E-06	2.76E-04	YES
19513	CG31267	-1.11	4.80E-07	5.91E-05	YES
27556	CG31681	-1.11	2.62E-05	6.49E-04	YES
1217	CG9466	-1.11	8.99E-05	1.49E-03	YES
22038	srw	-1.11	6.54E-04	6.09E-03	YES
14684	Obp49a	-1.10	6.45E-03	3.44E-02	YES
5056	jhamt	-1.10	1.08E-04	1.68E-03	YES
10862	bwa	-1.10	1.76E-07	3.78E-05	YES
27226	CG6776	-1.10	5.31E-05	1.05E-03	YES
4013	CG17633	-1.09	1.68E-04	2.29E-03	YES
9595	CG10550	-1.09	7.77E-08	2.94E-05	YES
16779	Glut4EF	-1.09	1.05E-05	3.60E-04	YES
6856	Glut4EF	-1.08	3.56E-05	7.97E-04	YES
15438	Orct2	-1.08	1.76E-04	2.37E-03	YES
4638	LysS	-1.08	2.74E-05	6.69E-04	YES
17777	CG18327	-1.08	9.30E-06	3.36E-04	YES
338	cher	-1.08	2.99E-03	1.90E-02	YES
6474	fon	-1.07	3.95E-08	2.36E-05	YES
15759	CG30047	-1.07	2.85E-07	4.62E-05	YES
14522	CG31266	-1.07	1.46E-04	2.09E-03	YES
20430	CG31974	-1.07	9.71E-05	1.57E-03	YES
29589	Obp99a	-1.07	2.72E-04	3.20E-03	YES
18835	CG30265	-1.06	2.26E-04	2.81E-03	YES
7932	CG18493	-1.06	6.00E-05	1.14E-03	YES
6914	CG34329	-1.06	1.93E-06	1.31E-04	YES
31581	Kr-h1	-1.06	1.48E-03	1.12E-02	YES
7202	CG11796	-1.06	2.64E-04	3.12E-03	YES
8377	inx3	-1.06	2.07E-07	4.09E-05	YES
17558	CG17633	-1.06	2.86E-04	3.33E-03	YES
8422	CG8628	-1.06	1.43E-04	2.07E-03	YES
19121	amos	-1.06	1.03E-03	8.57E-03	YES
28218	CG18269	-1.06	2.20E-04	2.76E-03	YES
17224	nimB3	-1.06	2.82E-04	3.29E-03	YES
17989	Yp2	-1.05	3.88E-06	1.92E-04	YES
13901	Prx2540-2	-1.05	5.62E-04	5.46E-03	YES
12852	CG4288	-1.05	2.78E-05	6.76E-04	YES
31704	bwa	-1.05	2.61E-06	1.53E-04	YES
5495	CG17119	-1.05	6.15E-05	1.16E-03	YES
21505	Jon65Aiii	-1.05	4.38E-05	9.22E-04	YES
20557	CG2930	-1.05	4.19E-05	8.96E-04	YES
15800	CG32695	-1.05	2.28E-05	5.95E-04	YES
26853	Nep2	-1.04	7.32E-05	1.30E-03	YES
8195	CG8562	-1.04	1.05E-05	3.61E-04	YES
24409	CG42249	-1.04	1.50E-03	1.14E-02	YES

28462	CG18327	-1.04	1.90E-05	5.24E-04	YES
1825	v	-1.04	1.14E-05	3.77E-04	YES
22	Act87E	-1.03	3.93E-06	1.93E-04	YES
23767	TpnC73F	-1.03	2.13E-06	1.39E-04	YES
19488	CG13912	-1.03	1.31E-04	1.93E-03	YES
24009	CG8661	-1.02	4.04E-05	8.76E-04	YES
2419	CG14949	-1.02	6.67E-03	3.52E-02	YES
9650	mag	-1.02	8.24E-05	1.41E-03	YES
5812	nimB3	-1.02	1.46E-04	2.09E-03	YES
16668	Obp49a	-1.02	1.16E-04	1.78E-03	YES
9988	LanB2	-1.01	2.41E-06	1.47E-04	YES
8149	CG9897	-1.01	2.53E-05	6.33E-04	YES
3354	nAcRbeta-21C	-1.01	3.10E-03	1.95E-02	YES
7060	CG8661	-1.01	1.27E-04	1.89E-03	YES
28312	CG18179	-1.01	9.69E-05	1.56E-03	YES
3128	CG13428	-1.01	3.48E-04	3.85E-03	YES
13633	CG31821	-1.01	2.09E-06	1.38E-04	YES
7032	CG13324	-1.00	6.11E-03	3.30E-02	YES
7616	CG4783	-1.00	1.29E-06	1.02E-04	YES
6698	CG8089	1.00	7.11E-08	2.94E-05	YES
12617	Rbf2	1.00	2.36E-05	6.05E-04	YES
21320	CG15601	1.00	8.11E-07	7.81E-05	YES
8212	dgt3	1.01	3.88E-07	5.34E-05	YES
12264	dah	1.01	6.84E-06	2.74E-04	YES
21921	CG8786	1.01	1.19E-06	9.93E-05	YES
30994	Sema-1a	1.01	1.12E-06	9.59E-05	YES
6047	CG1603	1.01	5.51E-06	2.39E-04	YES
29395	lola	1.01	5.17E-04	5.13E-03	YES
15137	CG8838	1.02	5.16E-05	1.03E-03	YES
2239	CG33331	1.02	3.25E-07	4.87E-05	YES
23470	tlk	1.02	3.81E-04	4.11E-03	YES
7164	insv	1.02	3.03E-07	4.74E-05	YES
14113	CG2662	1.02	9.78E-07	8.63E-05	YES
26842	mars	1.02	2.73E-07	4.54E-05	YES
25867	cnir	1.02	1.86E-06	1.29E-04	YES
13201	twe	1.02	7.13E-07	7.28E-05	YES
14219	CG6683	1.02	1.45E-07	3.65E-05	YES
10431	mei-218	1.02	2.91E-05	6.94E-04	YES
1963	CG12717	1.02	1.71E-04	2.31E-03	YES
25552	CG10669	1.02	1.12E-07	3.31E-05	YES
9812	msb1l	1.03	2.23E-06	1.42E-04	YES
1573	CG9641	1.03	9.12E-04	7.81E-03	YES
19892	CR43670	1.03	4.14E-05	8.90E-04	YES
9659	dap	1.03	9.27E-06	3.36E-04	YES
15239	CG14036	1.03	2.20E-07	4.14E-05	YES
5241	Gen	1.03	2.60E-05	6.47E-04	YES
445	sofe	1.03	6.89E-03	3.60E-02	YES
2170	CG14036	1.03	2.24E-04	2.80E-03	YES
29512	gammaTub37C	1.03	8.35E-06	3.15E-04	YES
18728	RnrL	1.03	8.14E-07	7.81E-05	YES
612	tef	1.03	8.85E-04	7.62E-03	YES
7242	CG6685	1.03	4.49E-07	5.75E-05	YES
6483	rt	1.03	3.44E-06	1.81E-04	YES
15053	CG4854	1.03	2.95E-04	3.40E-03	YES
26097	Hsp27	1.04	6.82E-06	2.74E-04	YES
2162	CG6928	1.04	6.05E-04	5.75E-03	YES
30961	E(var)3-9	1.04	2.52E-07	4.39E-05	YES
28375	fy	1.04	3.48E-07	5.07E-05	YES
27372	bcd	1.04	5.37E-04	5.28E-03	YES
5962	CG8152	1.04	1.79E-04	2.39E-03	YES
18819	qkr54B	1.04	5.27E-06	2.32E-04	YES
28230	CG3419	1.04	2.59E-04	3.09E-03	YES
11178	Su(var)2-10	1.04	6.42E-07	6.80E-05	YES
30791	CG14561	1.05	2.41E-07	4.30E-05	YES
18423	CG2924	1.05	2.25E-06	1.42E-04	YES
14194	CG13001	1.05	3.38E-07	4.98E-05	YES
14319	Fancd2	1.05	5.53E-07	6.31E-05	YES
8188	CG31457	1.06	4.02E-07	5.45E-05	YES
13044	nmdyn-D6	1.06	2.46E-06	1.49E-04	YES
31036	CG10638	1.06	4.97E-06	2.23E-04	YES

18250	Hsp27	1.06	2.19E-06	1.41E-04	YES
27402	Chd3	1.06	3.29E-05	7.59E-04	YES
20268	Rpn12R	1.06	9.01E-04	7.73E-03	YES
16489	CG11329	1.06	2.40E-06	1.47E-04	YES
8566	CG7101	1.07	2.36E-05	6.05E-04	YES
15045	spn-A	1.07	3.42E-06	1.80E-04	YES
15253	CG4730	1.07	1.65E-08	1.52E-05	YES
19500	CG12728	1.07	4.30E-06	2.03E-04	YES
16959	neur	1.07	5.54E-05	1.08E-03	YES
20336	mei-S332	1.07	7.11E-07	7.28E-05	YES
211	CG32318	1.08	1.60E-07	3.73E-05	YES
9181	CG1603	1.08	1.06E-06	9.21E-05	YES
3634	CG7650	1.09	3.37E-03	2.09E-02	YES
25044	CG6136	1.09	3.32E-08	2.10E-05	YES
7277	CG7130	1.09	4.16E-06	2.01E-04	YES
29104	CG42699	1.09	1.30E-06	1.03E-04	YES
2160	Gen	1.10	1.31E-04	1.93E-03	YES
28396	Oseg6	1.10	2.05E-08	1.67E-05	YES
11954	tum	1.10	8.28E-08	2.97E-05	YES
30374	Wnt5	1.10	2.21E-05	5.83E-04	YES
31710	Hsp27	1.10	4.18E-06	2.01E-04	YES
4685	CG3812	1.10	4.84E-07	5.91E-05	YES
3333	tef	1.11	6.29E-05	1.17E-03	YES
15785	IntS10	1.11	1.46E-08	1.47E-05	YES
15795	CG4089	1.11	2.37E-07	4.28E-05	YES
5897	CG32521	1.11	4.20E-06	2.01E-04	YES
4805	CTPsyn	1.11	8.05E-07	7.80E-05	YES
1654	CG6967	1.11	2.11E-04	2.68E-03	YES
19929	CG3975	1.11	6.45E-08	2.94E-05	YES
17526	CG10445	1.12	7.84E-07	7.71E-05	YES
7338	CG15643	1.12	1.65E-08	1.52E-05	YES
24241	CG7386	1.12	6.74E-08	2.94E-05	YES
27657	Cp110	1.12	3.59E-06	1.85E-04	YES
10393	dnk	1.12	1.64E-05	4.78E-04	YES
8130	CG15436	1.12	2.71E-08	1.92E-05	YES
174	CG31109	1.12	8.89E-05	1.48E-03	YES
21358	CycE	1.12	2.51E-03	1.67E-02	YES
457	CG4617	1.12	3.83E-05	8.41E-04	YES
7890	CG12713	1.13	1.36E-05	4.23E-04	YES
2176	Spc25	1.13	4.94E-08	2.65E-05	YES
26328	Klp67A	1.13	1.16E-06	9.84E-05	YES
2213	CG8180	1.13	5.40E-03	3.00E-02	YES
19886	CG14561	1.13	4.07E-06	1.98E-04	YES
12479	dnk	1.13	9.20E-06	3.35E-04	YES
8073	RnrS	1.13	8.32E-05	1.42E-03	YES
16164	Brf	1.13	4.13E-07	5.48E-05	YES
12243	CG7386	1.13	1.60E-07	3.73E-05	YES
28986	CG15047	1.13	2.28E-06	1.43E-04	YES
26273	CG31807	1.13	8.81E-07	8.18E-05	YES
31243	mus301	1.14	5.91E-07	6.43E-05	YES
8133	CoRest	1.14	6.20E-08	2.94E-05	YES
22462	CG33156	1.14	9.06E-06	3.32E-04	YES
10314	Spindly	1.14	1.96E-07	3.99E-05	YES
2720	Chrac-16	1.14	7.34E-07	7.42E-05	YES
27963	Hsp27	1.14	2.95E-06	1.64E-04	YES
25238	CG10336	1.15	4.88E-07	5.91E-05	YES
29932	Nek2	1.15	9.74E-07	8.63E-05	YES
8186	Cks30A	1.15	1.70E-04	2.31E-03	YES
28362	Sas-4	1.15	1.06E-07	3.26E-05	YES
18908	RhoGAP54D	1.16	2.09E-06	1.38E-04	YES
2412	Mcm3	1.16	1.58E-03	1.18E-02	YES
16796	Orc4	1.16	1.53E-06	1.17E-04	YES
1630	CG15387	1.16	3.20E-03	2.00E-02	YES
22534	Ctf4	1.16	8.91E-08	3.05E-05	YES
15641	Orc1	1.16	7.05E-05	1.27E-03	YES
32208	SAK	1.17	1.00E-06	8.80E-05	YES
2128	CG3430	1.17	3.55E-07	5.09E-05	YES
27801	CG14074	1.17	2.88E-06	1.61E-04	YES
27207	CG18011	1.17	9.55E-08	3.12E-05	YES
31358	CR43670	1.17	8.46E-07	7.95E-05	YES

12144	Spc105R	1.18	1.30E-07	3.46E-05	YES
7435	Spindly	1.18	1.16E-07	3.32E-05	YES
4859	Rad9	1.18	1.51E-05	4.53E-04	YES
3091	CG4570	1.18	2.77E-05	6.75E-04	YES
3923	thr	1.19	1.75E-07	3.78E-05	YES
21441	CG12702	1.19	3.64E-05	8.13E-04	YES
10537	CycE	1.19	3.40E-04	3.78E-03	YES
14104	trem	1.19	2.01E-05	5.45E-04	YES
17812	rt	1.19	2.07E-06	1.38E-04	YES
20824	CG30096	1.19	3.29E-05	7.58E-04	YES
23144	Hsp26	1.19	1.46E-06	1.13E-04	YES
6013	CG31251	1.20	6.00E-07	6.46E-05	YES
19210	spn-D	1.20	5.08E-05	1.02E-03	YES
1321	sti	1.20	2.77E-03	1.79E-02	YES
1969	CG11360	1.20	5.59E-07	6.31E-05	YES
9205	gd	1.20	1.10E-04	1.70E-03	YES
24160	CG42526	1.21	8.87E-08	3.05E-05	YES
13042	sas-6	1.21	3.25E-06	1.75E-04	YES
21471	CG31279	1.21	8.41E-08	2.98E-05	YES
25586	CG9902	1.21	1.24E-07	3.46E-05	YES
6287	CG34406	1.22	5.94E-06	2.51E-04	YES
28436	CG31053	1.22	3.60E-06	1.85E-04	YES
14881	CG3457	1.22	4.67E-05	9.58E-04	YES
30054	CG6752	1.22	1.76E-07	3.78E-05	YES
16098	CG3032	1.22	5.82E-07	6.43E-05	YES
2357	CG10050	1.23	5.77E-05	1.11E-03	YES
7509	CG7130	1.24	3.08E-06	1.68E-04	YES
15051	CG33213	1.24	2.62E-06	1.53E-04	YES
24177	CG5235	1.24	3.11E-06	1.69E-04	YES
22608	CG6425	1.24	2.23E-08	1.71E-05	YES
9208	CG11448	1.25	7.15E-08	2.94E-05	YES
28568	CG10011	1.25	3.11E-05	7.30E-04	YES
5978	CG5391	1.25	1.19E-05	3.86E-04	YES
29997	mus101	1.25	9.52E-07	8.52E-05	YES
12397	nmdyn-D7	1.26	3.57E-06	1.85E-04	YES
5763	Rpt3R	1.26	6.32E-05	1.17E-03	YES
5776	CG11360	1.26	6.28E-07	6.71E-05	YES
11914	CG12702	1.26	1.96E-05	5.35E-04	YES
29992	CG10445	1.27	2.13E-07	4.13E-05	YES
27993	Msh6	1.27	7.95E-07	7.74E-05	YES
4421	l(2)dtl	1.27	4.54E-06	2.10E-04	YES
32138	Orc5	1.27	1.53E-07	3.73E-05	YES
5157	CG33213	1.28	1.67E-07	3.78E-05	YES
8334	bora	1.28	2.98E-07	4.69E-05	YES
1246	Sodh-1	1.29	9.70E-05	1.56E-03	YES
31303	His4:CG33905	1.29	1.41E-05	4.37E-04	YES
718	CG5245	1.29	1.61E-05	4.73E-04	YES
19854	CG34406	1.29	1.09E-07	3.28E-05	YES
16972	CG6171	1.30	1.27E-07	3.46E-05	YES
9011	sas-6	1.30	1.94E-06	1.32E-04	YES
22910	Orc1	1.30	3.14E-07	4.79E-05	YES
2899	CG7730	1.30	9.10E-08	3.06E-05	YES
4534	nmdyn-D7	1.31	5.69E-08	2.82E-05	YES
22343	CG8247	1.31	4.33E-06	2.04E-04	YES
28268	Tsp96F	1.33	4.82E-07	5.91E-05	YES
17136	mei-38	1.33	4.41E-08	2.54E-05	YES
18333	CG31898	1.33	9.42E-09	1.42E-05	YES
8052	CG13690	1.33	1.15E-07	3.32E-05	YES
23347	Mis12	1.33	6.34E-07	6.74E-05	YES
12044	msb1l	1.33	3.20E-06	1.72E-04	YES
28336	CG31053	1.33	1.55E-07	3.73E-05	YES
19317	Klp61F	1.33	1.54E-06	1.17E-04	YES
7755	CycE	1.34	3.56E-07	5.09E-05	YES
20467	RhoGAP54D	1.34	6.39E-08	2.94E-05	YES
29664	Cks30A	1.35	8.16E-06	3.09E-04	YES
6851	pim	1.36	2.90E-08	1.94E-05	YES
2384	CG6012	1.36	9.77E-05	1.57E-03	YES
653	Msh6	1.36	3.08E-05	7.25E-04	YES
2218	CG5359	1.37	5.64E-05	1.09E-03	YES
562	CG12942	1.37	4.59E-03	2.67E-02	YES

7528	Orc5	1.38	2.91E-07	4.63E-05	YES
15551	Klp67A	1.38	2.72E-07	4.54E-05	YES
4170	CG13609	1.39	2.72E-09	9.75E-06	YES
20712	CG8247	1.39	1.65E-08	1.52E-05	YES
10059	ial	1.40	8.46E-07	7.95E-05	YES
9733	scra	1.40	1.76E-08	1.57E-05	YES
31954	CG32822	1.41	4.88E-08	2.65E-05	YES
11305	Orc2	1.41	1.11E-05	3.74E-04	YES
4376	CG13609	1.42	1.82E-05	5.10E-04	YES
5871	cid	1.42	1.00E-07	3.17E-05	YES
19122	CG12708	1.43	2.52E-07	4.39E-05	YES
32161	CG31279	1.43	7.66E-08	2.94E-05	YES
19672	CG14965	1.43	1.94E-07	3.98E-05	YES
10353	CG11164	1.44	4.50E-06	2.10E-04	YES
29306	msl-3	1.44	1.30E-05	4.12E-04	YES
24828	CG17658	1.45	1.02E-08	1.42E-05	YES
23227	Orc2	1.46	9.70E-08	3.12E-05	YES
2221	sip2	1.48	1.26E-03	9.95E-03	YES
22576	CG5245	1.49	1.73E-07	3.78E-05	YES
23653	CG32364	1.51	1.16E-06	9.83E-05	YES
30962	CG2990	1.54	1.34E-07	3.50E-05	YES
2094	CG30085	1.56	1.36E-03	1.06E-02	YES
22705	snRNA:U1:82Eb	1.57	7.17E-06	2.81E-04	YES
15153	CG34398	1.59	5.45E-06	2.38E-04	YES
4668	Poc1	1.59	1.29E-07	3.46E-05	YES
4506	pon	1.60	1.25E-07	3.46E-05	YES
3938	bam	1.60	9.68E-08	3.12E-05	YES
15747	CG8526	1.60	2.03E-08	1.67E-05	YES
13648	tobi	1.60	2.61E-07	4.47E-05	YES
163	Hmr	1.60	4.95E-06	2.23E-04	YES
31665	His1:CG33804	1.61	1.60E-06	1.18E-04	YES
26395	msd1	1.61	7.25E-07	7.34E-05	YES
10126	CG10013	1.62	1.55E-06	1.17E-04	YES
1097	Chrac-16	1.62	5.63E-04	5.47E-03	YES
24962	CG32364	1.63	3.32E-07	4.91E-05	YES
1836	Klp67A	1.66	7.07E-04	6.45E-03	YES
20415	mms4	1.66	4.67E-07	5.86E-05	YES
12221	Rad51D	1.68	3.17E-08	2.04E-05	YES
4320	His3:CG33830	1.75	2.24E-07	4.14E-05	YES
7070	Rad51D	1.75	9.19E-09	1.42E-05	YES
189	DNApol-alpha60	1.75	8.19E-05	1.40E-03	YES
26806	His3:CG31613	1.76	5.34E-07	6.21E-05	YES
27806	tobi	1.77	1.20E-08	1.44E-05	YES
20602	Try29F	1.81	2.51E-04	3.02E-03	YES
25900	Ipod	1.84	8.49E-09	1.42E-05	YES
26304	His3:CG31613	1.98	1.29E-06	1.02E-04	YES
27876	Orc2	2.01	3.15E-05	7.36E-04	YES
12094	CG14059	2.34	7.19E-09	1.42E-05	YES
25153	CG15263	2.37	1.12E-05	3.75E-04	YES
29437	snRNA:U12:73B	2.47	1.92E-06	1.31E-04	YES
30607	CG14059	2.49	2.29E-07	4.18E-05	YES
28010	snRNA:U11	2.74	1.67E-06	1.20E-04	YES
26382	CG34040	2.78	2.48E-05	6.26E-04	YES
3615	Ilp7	2.82	1.30E-10	1.05E-06	YES
14890	CG13091	3.55	3.35E-09	1.04E-05	YES

Table B4: Log fold change in transcript abundance due to mating in infected egg-producing females (Comparison D in Figure 5.1). This table contains all probes for genes that were significantly differentially expressed between virgin infected egg-producing females and mated infected egg-producing females.

ProbeUID	Gene name (where available)	logFC (Infected virgin - Infected mated)	p-value	B.H. Adj. p-value	Also significant in infected females?
26629	Vml	-6.33	6.58E-10	3.16E-06	NO
3102	CG31775	-3.94	1.35E-06	7.28E-05	NO
22213	CG31775	-2.59	4.36E-09	7.21E-06	NO
13478	CG9463	-2.50	9.54E-06	2.37E-04	NO
15190	LysB	-2.11	3.03E-03	1.62E-02	NO
28289	Osi19	-2.09	5.71E-04	4.46E-03	NO
15026	LysE	-1.98	3.48E-03	1.80E-02	NO
9035	CG13325	-1.96	1.86E-04	1.88E-03	NO
28953	CR40734	-1.92	8.87E-04	6.24E-03	NO
18183	CG8083	-1.89	3.31E-04	2.92E-03	NO
30409	term	-1.86	2.89E-03	1.57E-02	NO
5230	CG14120	-1.86	1.14E-06	6.68E-05	NO
20573	Obp19c	-1.86	8.78E-03	3.72E-02	NO
9092	CG13114	-1.80	3.24E-03	1.70E-02	NO
7411	CG31288	-1.78	3.67E-04	3.16E-03	NO
4222	LysD	-1.76	2.35E-03	1.33E-02	NO
27101	LysC	-1.71	2.06E-03	1.20E-02	NO
15130	yellow-g	-1.59	8.70E-03	3.70E-02	NO
20454	Obp99a	-1.58	1.77E-05	3.54E-04	NO
19692	Kr-h1	-1.58	1.48E-04	1.60E-03	NO
16013	snoRNA:Psi18S-1854b	-1.57	1.26E-03	8.16E-03	NO
8673	CG34203	-1.57	1.72E-05	3.49E-04	NO
23783	CG10834	-1.57	9.04E-05	1.12E-03	NO
10275	CG18585	-1.55	5.48E-07	4.55E-05	NO
11123	CG6296	-1.52	5.82E-03	2.69E-02	NO
5852	Odc1	-1.49	1.12E-06	6.60E-05	NO
26248	CG3036	-1.48	8.99E-06	2.29E-04	NO
2028	Jon66Cii	-1.47	3.58E-04	3.10E-03	NO
22518	CG10472	-1.46	3.54E-05	5.75E-04	NO
12718	CG1304	-1.45	1.38E-05	3.03E-04	NO
11503	CG42336	-1.45	6.11E-06	1.78E-04	NO
30107	Ser12	-1.44	1.10E-03	7.36E-03	NO
11721	CG13982	-1.41	1.80E-08	1.10E-05	NO
29349	Cg25C	-1.41	5.35E-03	2.51E-02	NO
10106	lambdaTry	-1.41	5.14E-07	4.36E-05	NO
31629	CG6738	-1.40	9.82E-07	6.28E-05	NO
15413	CG34137	-1.40	6.12E-03	2.80E-02	NO
2569	Mdr50	-1.38	2.38E-03	1.34E-02	NO
24295	Cyp6a21	-1.38	5.18E-04	4.15E-03	NO
5971	ninaE	-1.38	1.18E-04	1.35E-03	NO
31122	Mdr50	-1.37	2.28E-07	2.91E-05	NO
15671	CG1809	-1.36	1.01E-06	6.36E-05	NO
16942	bt	-1.36	1.10E-03	7.36E-03	NO
21292	CG17374	-1.34	3.41E-05	5.62E-04	NO
7839	Jon99Ci	-1.34	1.81E-04	1.84E-03	NO
6842	su(r)	-1.33	6.48E-05	8.78E-04	NO
20531	CG7968	-1.33	1.04E-05	2.51E-04	NO
7981	CG15745	-1.32	1.78E-06	8.58E-05	NO
10886	TpnC73F	-1.32	3.26E-07	3.57E-05	NO
10847	Scp2	-1.31	3.38E-07	3.61E-05	NO
11128	Npc2f	-1.31	1.55E-06	7.92E-05	NO
2251	CG17239	-1.30	7.18E-03	3.17E-02	NO
4813	Uhg5	-1.30	1.53E-07	2.51E-05	NO
2446	7SLRNA	-1.30	2.99E-03	1.61E-02	NO
10384	Drip	-1.29	5.49E-07	4.55E-05	NO
5260	CG2772	-1.29	8.98E-07	5.94E-05	NO
4711	su(r)	-1.29	6.58E-05	8.88E-04	NO
17981	Obp56d	-1.29	7.87E-07	5.48E-05	NO
25286	Vha100-5	-1.27	2.53E-05	4.56E-04	NO
22059	Ser12	-1.27	3.51E-05	5.70E-04	NO
10937	Glut4EF	-1.26	1.83E-06	8.69E-05	NO
27134	Ant2	-1.26	1.91E-06	8.83E-05	NO

1833	Npc1b	-1.26	3.51E-05	5.70E-04	NO
9371	Ppn	-1.25	1.59E-07	2.52E-05	NO
10323	CG7763	-1.25	1.79E-05	3.58E-04	NO
26979	CG31463	-1.25	1.25E-06	6.96E-05	NO
18753	Cyp313a1	-1.25	1.65E-06	8.19E-05	NO
29282	CG9286	-1.25	1.19E-05	2.74E-04	NO
9094	Cyp4d1	-1.25	1.21E-05	2.77E-04	NO
13813	CG3734	-1.25	3.31E-06	1.21E-04	NO
30111	bt	-1.24	1.13E-05	2.66E-04	NO
17295	CG10621	-1.24	1.08E-05	2.58E-04	NO
4047	CG14762	-1.24	2.02E-06	9.10E-05	NO
23500	Sox14	-1.23	7.18E-04	5.30E-03	NO
16368	trol	-1.23	1.14E-05	2.66E-04	NO
11785	CG10560	-1.23	2.30E-05	4.26E-04	NO
5625	CG6908	-1.22	1.80E-03	1.08E-02	NO
19828	CG14630	-1.22	8.11E-04	5.81E-03	NO
17405	Msp-300	-1.21	1.47E-05	3.16E-04	NO
8551	CG32407	-1.21	1.04E-07	2.04E-05	NO
9642	Phae1	-1.21	7.92E-05	1.01E-03	NO
28411	CG6206	-1.21	2.26E-07	2.91E-05	NO
13053	CG13155	-1.21	5.10E-05	7.40E-04	NO
17016	CG15155	-1.21	1.83E-05	3.63E-04	NO
29944	Cpr49Ab	-1.20	1.02E-05	2.48E-04	NO
2382	CG31248	-1.20	8.62E-05	1.08E-03	NO
26129	CG15201	-1.20	1.56E-07	2.51E-05	NO
5175	CG15155	-1.19	2.17E-04	2.11E-03	NO
10105	CG32407	-1.19	2.29E-07	2.91E-05	NO
29111	Jon65Ai	-1.19	2.38E-05	4.36E-04	NO
20476	PGRP-LB	-1.19	1.52E-04	1.63E-03	NO
13768	Hrb98DE	-1.19	7.53E-07	5.33E-05	NO
19652	wdp	-1.18	3.45E-05	5.64E-04	NO
25211	CG9498	-1.18	1.75E-04	1.81E-03	NO
10817	trol	-1.18	1.11E-05	2.61E-04	NO
2043	CG9672	-1.18	4.36E-05	6.62E-04	NO
11182	Ndg	-1.17	9.96E-04	6.81E-03	NO
30783	CG31463	-1.17	5.89E-05	8.17E-04	NO
29204	CG31810	-1.17	2.34E-04	2.24E-03	NO
13409	CG5804	-1.17	1.06E-05	2.54E-04	NO
12443	Hsromega	-1.17	1.15E-03	7.62E-03	NO
21478	CG9673	-1.16	2.24E-05	4.16E-04	NO
27591	CG3734	-1.16	1.99E-05	3.83E-04	NO
29017	CG9286	-1.16	4.66E-05	6.92E-04	NO
6316	CG10131	-1.16	1.37E-05	3.02E-04	NO
4584	CG31265	-1.16	1.66E-04	1.74E-03	NO
17516	CG10253	-1.16	2.96E-04	2.69E-03	NO
14367	CG13712	-1.16	5.31E-05	7.62E-04	NO
27244	IM10	-1.16	1.36E-03	8.67E-03	NO
27411	yellow-d2	-1.15	6.26E-03	2.85E-02	NO
25362	CG14777	-1.15	6.05E-06	1.77E-04	NO
17562	CG10827	-1.15	1.40E-04	1.53E-03	NO
12879	CG32687	-1.15	1.01E-05	2.46E-04	NO
16189	CG6048	-1.14	3.04E-04	2.73E-03	NO
12805	CG8740	-1.14	5.39E-07	4.53E-05	NO
1815	Oscillin	-1.14	2.47E-06	1.04E-04	NO
13336	CG32091	-1.14	3.07E-06	1.17E-04	NO
19507	CG15096	-1.14	4.41E-04	3.65E-03	NO
12285	CG10345	-1.13	2.51E-05	4.53E-04	NO
19612	CG43078	-1.13	4.56E-06	1.48E-04	NO
19995	Cyp4g1	-1.13	8.46E-04	6.00E-03	NO
3806	CG13868	-1.13	2.64E-05	4.67E-04	NO
13812	CG7142	-1.13	2.66E-05	4.68E-04	NO
3523	CG31975	-1.13	9.37E-05	1.16E-03	NO
28885	CG17597	-1.12	4.69E-04	3.84E-03	NO
4600	proPO-A1	-1.12	3.81E-06	1.32E-04	NO
8677	CG15358	-1.12	2.12E-05	4.02E-04	NO
30799	vkg	-1.12	3.00E-06	1.15E-04	NO
606	CG2781	-1.12	1.70E-03	1.03E-02	NO
22727	CG11378	-1.12	8.84E-05	1.10E-03	NO
21219	Cpr49Ab	-1.12	1.58E-05	3.31E-04	NO
4528	CG14490	-1.12	6.49E-04	4.92E-03	NO

19508	CG10345	-1.11	3.09E-05	5.23E-04	NO
17100	Cg25C	-1.11	1.96E-08	1.10E-05	NO
21972	Cyp4d1	-1.11	1.11E-05	2.61E-04	NO
9539	Dp1	-1.11	5.06E-08	1.60E-05	NO
12684	IM4	-1.11	8.76E-03	3.72E-02	NO
20699	Drs-l	-1.11	4.31E-04	3.59E-03	NO
26919	CG14661	-1.11	3.66E-07	3.75E-05	NO
14608	Spn6	-1.11	4.70E-05	6.96E-04	NO
20241	Mf	-1.10	2.04E-03	1.19E-02	NO
29202	CG32425	-1.10	9.18E-06	2.31E-04	NO
28859	Cyp312a1	-1.10	5.77E-05	8.07E-04	NO
11478	CG14661	-1.10	1.28E-07	2.32E-05	NO
30313	CG15534	-1.09	2.61E-05	4.64E-04	NO
20953	CG2254	-1.09	1.79E-05	3.58E-04	NO
15304	CG3814	-1.09	1.17E-03	7.71E-03	NO
20328	CG32483	-1.09	3.05E-05	5.18E-04	NO
6880	CG6048	-1.09	6.20E-03	2.83E-02	NO
10712	CG9394	-1.09	2.77E-06	1.11E-04	NO
12000	CG17646	-1.09	3.43E-06	1.24E-04	NO
30277	CG1246	-1.08	7.06E-05	9.37E-04	NO
2748	CG34329	-1.08	5.78E-05	8.07E-04	NO
4838	Cpr49Ae	-1.08	2.15E-04	2.09E-03	NO
21902	laza	-1.08	7.21E-07	5.24E-05	NO
8255	inx2	-1.08	5.26E-06	1.61E-04	NO
27845	CG6900	-1.08	4.75E-05	7.01E-04	NO
22808	CG17664	-1.08	2.59E-03	1.44E-02	NO
8183	Oatp58Dc	-1.07	2.05E-05	3.92E-04	NO
15468	nimC1	-1.07	1.12E-04	1.30E-03	NO
9956	CG1143	-1.07	2.13E-05	4.02E-04	NO
4005	CG32425	-1.07	1.21E-05	2.77E-04	NO
18551	CG31150	-1.07	5.09E-06	1.59E-04	NO
5787	dsx	-1.07	2.37E-04	2.26E-03	NO
13413	CG13912	-1.07	4.20E-05	6.45E-04	NO
21288	Mhc	-1.07	7.80E-06	2.08E-04	NO
13860	Spn28D	-1.07	1.91E-04	1.92E-03	NO
29848	CG3097	-1.07	8.40E-06	2.19E-04	NO
3165	CG8299	-1.07	1.38E-03	8.76E-03	NO
10851	CG7203	-1.07	9.67E-05	1.18E-03	NO
2065	CG9394	-1.07	1.74E-05	3.51E-04	NO
27248	CG31777	-1.07	7.13E-06	1.95E-04	NO
15087	Oatp58Db	-1.06	3.64E-05	5.84E-04	NO
14549	dally	-1.06	1.06E-06	6.47E-05	NO
20959	pcl	-1.06	4.58E-04	3.77E-03	NO
29717	up	-1.06	2.64E-06	1.08E-04	NO
16961	Cg25C	-1.06	1.38E-05	3.03E-04	NO
10703	Vha100-5	-1.06	1.55E-06	7.92E-05	NO
27815	Pif1A	-1.06	8.89E-04	6.24E-03	NO
14091	CG15353	-1.06	5.39E-06	1.64E-04	NO
12820	CG3706	-1.05	1.82E-03	1.09E-02	NO
18298	CG12825	-1.05	5.65E-06	1.69E-04	NO
17347	CG9005	-1.05	2.06E-04	2.03E-03	NO
6016	SC35	-1.05	3.69E-06	1.29E-04	NO
2929	Ser6	-1.05	3.35E-04	2.94E-03	NO
8604	CG5527	-1.05	5.71E-06	1.70E-04	NO
12163	CG15628	-1.05	4.48E-07	4.19E-05	NO
24116	sty	-1.05	9.57E-06	2.37E-04	NO
26650	trol	-1.05	8.12E-06	2.15E-04	NO
30971	Tm2	-1.05	4.51E-05	6.77E-04	NO
16956	pan	-1.05	1.74E-03	1.05E-02	NO
15351	CG16799	-1.05	4.87E-06	1.53E-04	NO
8907	CHKov1	-1.04	1.60E-03	9.86E-03	NO
951	CG31086	-1.04	1.27E-05	2.86E-04	NO
3101	CG3857	-1.04	2.19E-05	4.09E-04	NO
17803	CG6067	-1.04	3.83E-05	6.03E-04	NO
15164	Cyp6a2	-1.04	3.62E-03	1.86E-02	NO
20956	Obp57c	-1.04	3.76E-04	3.22E-03	NO
21577	Neu2	-1.04	1.20E-02	4.77E-02	NO
9510	CG13428	-1.04	2.07E-05	3.94E-04	NO
7781	CG17664	-1.04	4.35E-03	2.13E-02	NO
7394	ps	-1.04	9.57E-07	6.19E-05	NO

8721	Cpr62Bb	-1.04	1.59E-06	8.02E-05	NO
20295	CG13737	-1.03	5.93E-03	2.73E-02	NO
26411	Unc-89	-1.03	2.22E-06	9.65E-05	NO
25942	CG11458	-1.03	5.02E-07	4.34E-05	NO
21909	Mhc	-1.03	4.86E-06	1.53E-04	NO
21988	BM-40-SPARC	-1.03	2.36E-05	4.33E-04	NO
30126	CG3246	-1.03	1.00E-06	6.35E-05	NO
5696	CG7296	-1.03	2.80E-05	4.89E-04	NO
13618	CG14111	-1.03	6.25E-03	2.85E-02	NO
16452	CG31198	-1.03	3.44E-05	5.64E-04	NO
16572	CG17477	-1.03	1.49E-05	3.18E-04	NO
13375	HmgZ	-1.03	6.16E-05	8.44E-04	NO
16891	Uhg2	-1.03	2.53E-07	3.03E-05	NO
1415	CG14687	-1.03	3.92E-06	1.34E-04	NO
21062	CG34291	-1.03	1.35E-03	8.63E-03	NO
23893	dp	-1.03	3.28E-06	1.21E-04	NO
3028	Cpr67B	-1.02	6.71E-07	5.06E-05	NO
18289	PGRP-SC1a	-1.02	4.72E-04	3.85E-03	NO
15454	Pvr	-1.02	7.92E-07	5.49E-05	NO
30824	CG3097	-1.02	2.18E-05	4.08E-04	NO
5510	Gs2	-1.02	1.75E-04	1.81E-03	NO
26135	Cyp4d21	-1.02	4.97E-05	7.27E-04	NO
7786	bt	-1.02	8.80E-03	3.73E-02	NO
15244	dally	-1.02	1.44E-06	7.59E-05	NO
22939	Phk-3	-1.02	1.86E-06	8.75E-05	NO
12555	dom	-1.02	1.00E-04	1.21E-03	NO
26326	CG15406	-1.01	1.97E-05	3.81E-04	NO
13985	SPR	-1.01	3.87E-04	3.29E-03	NO
23828	Vha100-4	-1.01	4.78E-05	7.03E-04	NO
10834	Ser6	-1.01	6.21E-03	2.83E-02	NO
22620	CG14963	-1.01	1.71E-05	3.48E-04	NO
4095	CG10365	-1.01	3.34E-07	3.59E-05	NO
16946	nemy	-1.01	7.02E-05	9.33E-04	NO
27542	Ca-P60A	-1.01	4.32E-07	4.12E-05	NO
2480	RpL36	-1.01	1.89E-03	1.12E-02	NO
9699	ImpL2	-1.01	4.80E-06	1.52E-04	NO
29272	Obp56e	-1.01	3.69E-05	5.89E-04	NO
32032	Peritrophin-15b	-1.01	1.57E-03	9.71E-03	NO
21640	sad	-1.01	2.37E-06	1.01E-04	NO
19646	Pmp70	-1.01	4.01E-04	3.39E-03	NO
16924	CG14629	-1.01	4.07E-04	3.43E-03	NO
13414	CG14968	-1.01	3.60E-05	5.80E-04	NO
7227	Iris	-1.00	3.47E-06	1.25E-04	NO
19591	CG6602	-1.00	2.66E-04	2.48E-03	NO
22242	CG15126	-1.00	1.94E-03	1.15E-02	NO
14677	CG34194	-1.00	1.16E-03	7.63E-03	NO
4815	CG3523	-1.00	7.98E-05	1.02E-03	NO
15744	CG10116	-1.00	6.22E-05	8.50E-04	NO
10822	pcl	-1.00	6.12E-04	4.72E-03	NO
30217	CG2022	-1.00	2.01E-05	3.85E-04	NO
22634	CG32702	-1.00	9.90E-07	6.31E-05	NO
10198	mst	1.00	5.36E-04	4.25E-03	NO
10389	gkt	1.00	4.04E-06	1.37E-04	NO
1559	CG11023	1.00	1.09E-03	7.28E-03	NO
29309	exd	1.00	5.70E-03	2.65E-02	NO
30000	CG4496	1.00	1.43E-06	7.56E-05	NO
18274	cdc2	1.00	1.04E-06	6.47E-05	NO
8459	CG42678	1.00	1.09E-06	6.54E-05	NO
3329	CG10543	1.00	7.62E-08	1.85E-05	NO
6963	S6kII	1.00	9.21E-05	1.14E-03	NO
13107	twe	1.00	2.72E-07	3.21E-05	NO
1923	scra	1.01	2.49E-05	4.51E-04	NO
4073	Nipped-B	1.01	5.70E-05	8.01E-04	NO
901	RhoGAP71E	1.01	2.65E-05	4.68E-04	NO
380	CG9727	1.01	1.02E-02	4.20E-02	NO
18177	CG30343	1.01	1.73E-05	3.49E-04	NO
30692	CR42871	1.01	1.06E-05	2.54E-04	NO
2331	oaf	1.01	4.54E-03	2.20E-02	NO
29048	cuff	1.01	9.94E-07	6.31E-05	NO
25987	ftz-f1	1.01	1.84E-05	3.63E-04	NO

4701	CG3313	1.01	1.16E-04	1.34E-03	NO
13851	Sse	1.01	1.32E-05	2.94E-04	NO
10475	CG6791	1.01	7.63E-04	5.55E-03	NO
18984	CG3225	1.01	3.70E-07	3.75E-05	NO
21581	CG13430	1.01	3.62E-05	5.82E-04	NO
5266	nAcRalpha-80B	1.01	2.08E-07	2.78E-05	NO
12582	qua	1.02	2.92E-07	3.36E-05	NO
29840	mthl1	1.02	1.30E-05	2.91E-04	NO
13708	CG42351	1.02	1.12E-04	1.30E-03	NO
21403	Caf1	1.02	1.41E-03	8.89E-03	NO
18110	homer	1.02	7.11E-05	9.41E-04	NO
19907	trk	1.02	6.70E-07	5.06E-05	NO
27566	CG7102	1.02	4.18E-05	6.43E-04	NO
13693	Ssb-c31a	1.02	4.97E-06	1.56E-04	NO
5987	CG15653	1.02	1.67E-05	3.41E-04	NO
27392	Corp	1.02	2.99E-03	1.61E-02	NO
4331	CG33214	1.02	3.90E-06	1.34E-04	NO
3310	tefu	1.02	8.93E-03	3.77E-02	NO
25524	CG14562	1.02	2.25E-06	9.76E-05	NO
28459	mus304	1.02	8.84E-05	1.11E-03	NO
23346	lat	1.02	2.33E-04	2.23E-03	NO
25048	Ephrin	1.02	5.24E-06	1.61E-04	NO
31907	ssp	1.02	1.36E-04	1.50E-03	NO
27113	CG33969	1.02	2.03E-04	2.01E-03	NO
19839	mei-W68	1.03	4.74E-05	7.00E-04	NO
29064	CG3679	1.03	7.91E-03	3.43E-02	NO
17937	CG4497	1.03	2.88E-05	4.99E-04	NO
27171	CG13032	1.03	4.14E-03	2.06E-02	NO
30333	lola	1.03	7.09E-05	9.39E-04	NO
6715	CG11873	1.03	9.62E-06	2.38E-04	NO
20453	how	1.03	3.27E-04	2.88E-03	NO
19133	CG7208	1.03	4.80E-07	4.32E-05	NO
24077	CkIIalpha-i1	1.03	2.81E-06	1.12E-04	NO
31124	CG8679	1.03	4.30E-06	1.42E-04	NO
8905	Takl1	1.03	6.67E-04	5.02E-03	NO
14745	CG17186	1.03	1.05E-06	6.47E-05	NO
12084	zwilch	1.03	2.65E-06	1.08E-04	NO
15494	hb	1.03	9.23E-06	2.32E-04	NO
14590	RhoGEF4	1.03	7.38E-06	2.00E-04	NO
11749	CG30183	1.03	3.00E-05	5.13E-04	NO
6003	CG13876	1.03	3.09E-05	5.23E-04	NO
23065	Taspase1	1.04	2.10E-06	9.33E-05	NO
15065	CG7341	1.04	4.94E-08	1.59E-05	NO
3474	CG3085	1.04	9.13E-06	2.31E-04	NO
4885	WRNexo	1.04	2.27E-06	9.82E-05	NO
757	gus	1.04	1.27E-03	8.19E-03	NO
1215	hd	1.04	9.92E-05	1.20E-03	NO
15214	vret	1.04	1.05E-07	2.04E-05	NO
21851	LBR	1.04	5.73E-04	4.48E-03	NO
29572	CG9203	1.04	1.04E-04	1.24E-03	NO
28301	CG14535	1.04	1.08E-04	1.27E-03	NO
24836	CG3326	1.04	5.48E-06	1.65E-04	NO
482	CG11486	1.04	7.17E-03	3.17E-02	NO
19195	CG4935	1.04	5.99E-08	1.69E-05	NO
2647	CG3995	1.04	9.17E-05	1.14E-03	NO
8127	CG18171	1.04	1.12E-02	4.52E-02	NO
14444	CG17490	1.05	2.97E-07	3.39E-05	NO
16242	CG3238	1.05	2.53E-08	1.15E-05	NO
3501	Ptp99A	1.05	1.53E-04	1.64E-03	NO
15646	NKAIN	1.05	4.47E-04	3.69E-03	NO
12612	CG7609	1.05	1.14E-07	2.15E-05	NO
10109	Pli	1.05	1.51E-05	3.21E-04	NO
100	ird1	1.05	9.58E-03	3.99E-02	NO
2271	l(1)G0232	1.05	6.51E-03	2.94E-02	NO
30713	magu	1.05	5.58E-04	4.38E-03	NO
25253	CG4617	1.05	4.98E-07	4.32E-05	NO
18043	CG7130	1.05	5.42E-06	1.64E-04	NO
20735	CG7208	1.05	3.44E-07	3.65E-05	NO
29783	Caf1-105	1.05	3.09E-07	3.45E-05	NO
7980	CG12766	1.05	1.66E-03	1.01E-02	NO

8260	CG2941	1.05	9.61E-08	2.00E-05	NO
28160	CG32043	1.05	1.41E-03	8.93E-03	NO
22588	Psf1	1.05	2.49E-07	2.99E-05	NO
3863	wus	1.05	4.30E-03	2.12E-02	NO
26641	CG42233	1.05	4.02E-06	1.37E-04	NO
7268	CycA	1.05	1.40E-06	7.45E-05	NO
4023	CG6325	1.05	7.27E-07	5.26E-05	NO
13484	CG10866	1.06	9.73E-06	2.40E-04	NO
857	qkr58E-2	1.06	9.02E-03	3.80E-02	NO
9038	WRNexo	1.06	1.25E-06	6.96E-05	NO
8475	Cyp6v1	1.06	3.87E-05	6.07E-04	NO
25071	hang	1.06	9.82E-04	6.72E-03	NO
2633	Spt20	1.06	1.70E-03	1.03E-02	NO
24070	Ercc1	1.06	4.22E-08	1.46E-05	NO
24140	Det	1.06	1.83E-04	1.87E-03	NO
2533	CG8159	1.06	6.53E-07	5.00E-05	NO
16236	CG12077	1.06	7.92E-06	2.11E-04	NO
25984	pea	1.06	2.71E-04	2.51E-03	NO
19638	CG5144	1.06	2.15E-05	4.04E-04	NO
15651	CG33096	1.06	2.47E-04	2.34E-03	NO
17551	Orc6	1.06	1.58E-06	8.00E-05	NO
2931	Taspase1	1.06	1.33E-04	1.47E-03	NO
17775	CG1663	1.06	1.13E-07	2.15E-05	NO
11859	CG6540	1.07	1.39E-05	3.03E-04	NO
832	CG8405	1.07	5.13E-06	1.59E-04	NO
23397	APC4	1.07	1.54E-04	1.65E-03	NO
20806	Mtl	1.07	1.18E-03	7.79E-03	NO
12527	CG7192	1.07	9.34E-04	6.48E-03	NO
2152	cos	1.07	1.98E-04	1.97E-03	NO
8829	CG11141	1.07	8.68E-06	2.24E-04	NO
26439	CG2924	1.07	1.28E-05	2.88E-04	NO
5166	CG10638	1.07	3.59E-05	5.79E-04	NO
24071	Df31	1.07	2.54E-05	4.56E-04	NO
3378	Hen1	1.07	5.90E-03	2.72E-02	NO
21322	Cyp6a19	1.07	6.42E-06	1.83E-04	NO
8594	CG15653	1.07	2.12E-05	4.02E-04	NO
7893	CG42248	1.07	3.25E-05	5.44E-04	NO
6214	CG10147	1.07	9.55E-05	1.17E-03	NO
1788	CG13896	1.07	1.34E-07	2.40E-05	NO
1596	dgt5	1.07	1.61E-06	8.11E-05	NO
21420	dco	1.07	2.37E-06	1.01E-04	NO
7390	Ada	1.07	5.53E-06	1.66E-04	NO
26451	lqfR	1.07	6.32E-06	1.81E-04	NO
5896	CG6685	1.07	2.37E-04	2.26E-03	NO
28845	CG2061	1.08	3.12E-06	1.18E-04	NO
5101	tan	1.08	1.05E-06	6.47E-05	NO
2965	CG12299	1.08	6.36E-03	2.89E-02	NO
7370	dnt	1.08	6.01E-06	1.76E-04	NO
28135	mute	1.08	1.04E-04	1.24E-03	NO
22703	CG9418	1.08	4.40E-05	6.67E-04	NO
8379	DAAM	1.08	2.91E-04	2.65E-03	NO
17213	Gef26	1.08	1.08E-04	1.27E-03	NO
8954	mbo	1.08	7.00E-07	5.16E-05	NO
19017	CG8944	1.08	3.31E-06	1.21E-04	NO
18111	CG30381	1.09	5.46E-06	1.65E-04	NO
29623	CG3919	1.09	4.12E-07	4.00E-05	NO
19677	CG33331	1.09	1.04E-07	2.04E-05	NO
16468	CG4996	1.09	3.07E-04	2.76E-03	NO
27081	CG7192	1.09	1.29E-04	1.44E-03	NO
14325	Su(H)	1.10	1.09E-06	6.54E-05	NO
17009	CG9272	1.10	2.82E-05	4.91E-04	NO
13845	CG31849	1.10	3.66E-07	3.75E-05	NO
30265	CG17343	1.10	6.62E-07	5.04E-05	NO
8618	CG17803	1.10	6.34E-06	1.82E-04	NO
29711	CG42797	1.10	2.97E-04	2.69E-03	NO
5390	mol	1.10	3.40E-06	1.23E-04	NO
25410	Mal-A8	1.10	8.98E-05	1.12E-03	NO
25159	mei-S332	1.10	1.91E-06	8.83E-05	NO
24277	Rab23	1.10	9.02E-06	2.29E-04	NO
5190	Hr39	1.10	7.80E-04	5.65E-03	NO

1374	CG4363	1.10	6.66E-03	2.99E-02	NO
4926	CG42676	1.10	2.74E-04	2.53E-03	NO
1741	CG10914	1.10	1.22E-04	1.39E-03	NO
779	mod(mdg4)	1.10	1.06E-04	1.26E-03	NO
31749	Su(var)3-9	1.11	6.46E-06	1.84E-04	NO
31846	CG11023	1.11	2.12E-06	9.38E-05	NO
19286	CG15653	1.11	5.80E-05	8.08E-04	NO
28538	mud	1.11	8.24E-06	2.16E-04	NO
496	CG12391	1.11	1.63E-05	3.37E-04	NO
22413	gammaTub37C	1.11	1.06E-06	6.47E-05	NO
22918	CG7441	1.11	7.01E-06	1.93E-04	NO
4191	Fmr1	1.11	3.80E-08	1.38E-05	NO
20673	CG11983	1.11	2.06E-04	2.03E-03	NO
4604	D19A	1.11	3.73E-07	3.75E-05	NO
548	CG17186	1.11	3.12E-04	2.79E-03	NO
8436	cutlet	1.11	2.06E-05	3.92E-04	NO
27779	slx1	1.11	1.91E-07	2.73E-05	NO
19707	CG7650	1.12	4.50E-07	4.20E-05	NO
2893	CG34149	1.12	2.77E-07	3.24E-05	NO
84	Ssl1	1.12	3.18E-04	2.83E-03	NO
28678	bs	1.12	5.15E-06	1.60E-04	NO
2115	trsn	1.12	1.79E-04	1.83E-03	NO
22207	CG16863	1.12	3.29E-08	1.29E-05	NO
18644	CG42254	1.12	3.25E-06	1.21E-04	NO
3569	CG14803	1.12	2.09E-03	1.21E-02	NO
28200	smg	1.12	2.30E-04	2.20E-03	NO
22871	Ptp61F	1.12	3.13E-04	2.79E-03	NO
23871	CG30059	1.12	7.07E-05	9.38E-04	NO
19193	Sry-beta	1.12	3.02E-05	5.16E-04	NO
12509	CG17233	1.13	1.58E-06	8.00E-05	NO
19486	sn	1.13	8.38E-05	1.06E-03	NO
23742	wech	1.13	6.86E-04	5.12E-03	NO
24588	CG1832	1.13	2.43E-03	1.37E-02	NO
24045	Kebab	1.13	9.44E-06	2.35E-04	NO
10212	CG12179	1.13	6.68E-03	3.00E-02	NO
21655	CG11436	1.13	2.13E-07	2.81E-05	NO
8836	CG12662	1.13	1.63E-05	3.36E-04	NO
18153	CycB3	1.14	4.15E-04	3.48E-03	NO
16498	Rad51C	1.14	9.17E-06	2.31E-04	NO
14225	brat	1.14	7.72E-04	5.60E-03	NO
17065	rap	1.14	1.19E-06	6.82E-05	NO
25964	borr	1.14	3.91E-06	1.34E-04	NO
16832	CG12567	1.14	3.90E-03	1.96E-02	NO
4811	CG5664	1.14	7.05E-08	1.81E-05	NO
9365	orb2	1.14	4.11E-03	2.04E-02	NO
23291	CG6808	1.15	1.39E-08	1.06E-05	NO
2105	CG5144	1.15	3.47E-06	1.25E-04	NO
4012	mod(mdg4)	1.15	3.47E-08	1.32E-05	NO
18708	CG32786	1.15	3.10E-07	3.45E-05	NO
23781	CG12077	1.15	4.47E-05	6.73E-04	NO
5135	Mical	1.15	1.38E-08	1.06E-05	NO
12306	CG13088	1.15	4.48E-09	7.21E-06	NO
30870	CG10880	1.16	2.06E-07	2.77E-05	NO
27890	Ripalpha	1.16	8.43E-05	1.06E-03	NO
7191	CG17361	1.16	9.19E-08	1.95E-05	NO
14235	CG31875	1.16	8.40E-08	1.88E-05	NO
13772	CkIIalpha-i1	1.16	6.21E-07	4.87E-05	NO
7344	exu	1.16	6.81E-07	5.07E-05	NO
1157	CG14962	1.16	2.48E-06	1.04E-04	NO
25655	CG18262	1.16	9.95E-05	1.21E-03	NO
6020	CG7156	1.16	2.01E-06	9.09E-05	NO
11418	CG12155	1.16	3.19E-04	2.83E-03	NO
27015	blos4	1.16	3.46E-05	5.66E-04	NO
10025	slx1	1.16	4.32E-09	7.21E-06	NO
22231	CG7110	1.16	2.27E-04	2.19E-03	NO
4897	CG34252	1.17	7.74E-06	2.07E-04	NO
23548	CG11727	1.17	4.28E-05	6.54E-04	NO
28966	fzy	1.17	1.14E-04	1.32E-03	NO
1644	CG1737	1.17	5.29E-03	2.48E-02	NO
2868	tgo	1.17	3.34E-04	2.93E-03	NO

3858	neur	1.17	2.82E-04	2.59E-03	NO
28599	CG10979	1.17	5.30E-06	1.62E-04	NO
23847	ash1	1.17	1.10E-05	2.60E-04	NO
25966	CG31122	1.17	1.74E-04	1.80E-03	NO
22485	CG30381	1.17	6.15E-05	8.42E-04	NO
31298	ial	1.17	3.45E-04	3.00E-03	NO
25300	Rbf2	1.18	6.80E-07	5.07E-05	NO
18885	CG43759	1.18	4.26E-08	1.46E-05	NO
6180	CG14102	1.18	3.67E-08	1.35E-05	NO
29832	CG15484	1.18	1.71E-07	2.62E-05	NO
20036	Bx	1.18	2.96E-08	1.24E-05	NO
6421	CG17802	1.18	6.45E-07	4.97E-05	NO
14125	CG11750	1.19	3.04E-06	1.16E-04	NO
3289	gkt	1.19	1.23E-02	4.88E-02	NO
1178	5Ptasel	1.19	1.43E-03	9.01E-03	NO
14728	mute	1.19	1.27E-04	1.43E-03	NO
17152	CG4174	1.19	1.53E-05	3.23E-04	NO
17512	Sirt6	1.19	2.06E-08	1.10E-05	NO
20931	CG13142	1.19	3.49E-05	5.69E-04	NO
7608	CG3038	1.19	1.33E-05	2.96E-04	NO
1555	CG10147	1.19	3.03E-03	1.62E-02	NO
2630	CG4676	1.20	3.32E-04	2.92E-03	NO
28060	CG32783	1.20	2.16E-05	4.05E-04	NO
4187	lack	1.20	1.10E-03	7.36E-03	NO
3068	CBP	1.20	2.37E-05	4.33E-04	NO
1800	Lap1	1.20	7.86E-04	5.68E-03	NO
21455	Wnt5	1.20	1.04E-04	1.24E-03	NO
26310	CG5877	1.21	1.76E-05	3.53E-04	NO
1723	jvl	1.21	1.53E-03	9.49E-03	NO
2730	Bgb	1.21	9.23E-03	3.87E-02	NO
30491	CG15387	1.22	2.57E-05	4.60E-04	NO
5972	CG13005	1.22	6.45E-06	1.84E-04	NO
24253	brat	1.22	1.20E-05	2.75E-04	NO
1442	CG12129	1.22	2.69E-03	1.48E-02	NO
7146	spel1	1.22	1.51E-05	3.21E-04	NO
1297	CG30343	1.22	1.18E-03	7.77E-03	NO
24733	gfzf	1.22	1.25E-06	6.96E-05	NO
4517	mei-P22	1.23	1.09E-05	2.59E-04	NO
1743	CG17440	1.23	1.50E-04	1.61E-03	NO
19395	CG13749	1.24	1.18E-05	2.73E-04	NO
26711	CG18769	1.24	2.00E-04	1.99E-03	NO
26327	CG43144	1.24	5.72E-05	8.03E-04	NO
27820	CoRest	1.24	1.12E-07	2.15E-05	NO
9994	ash1	1.24	5.01E-05	7.31E-04	NO
32148	CG7504	1.25	2.64E-06	1.08E-04	NO
16449	Mal-A8	1.25	4.26E-08	1.46E-05	NO
2756	CG14446	1.25	6.29E-07	4.91E-05	NO
16701	CG2023	1.25	1.39E-05	3.04E-04	NO
3797	CG31251	1.26	8.95E-03	3.78E-02	NO
10898	mol	1.26	7.09E-04	5.25E-03	NO
1667	CG13876	1.26	1.20E-02	4.78E-02	NO
11456	CG32100	1.26	1.18E-04	1.35E-03	NO
7094	CG16863	1.27	5.28E-08	1.62E-05	NO
27827	CG13896	1.27	1.92E-05	3.75E-04	NO
3321	CG12253	1.28	8.65E-05	1.09E-03	NO
4471	CG5664	1.28	5.41E-08	1.64E-05	NO
9941	Mal-B1	1.28	1.84E-05	3.63E-04	NO
14352	ptip	1.28	1.40E-04	1.54E-03	NO
21264	exu	1.28	1.85E-06	8.71E-05	NO
29976	yrt	1.29	2.96E-06	1.15E-04	NO
9849	CR43257	1.30	2.26E-07	2.91E-05	NO
554	Pbp95	1.30	6.82E-04	5.10E-03	NO
12962	CG6985	1.31	3.95E-04	3.35E-03	NO
31656	CG34406	1.31	1.25E-04	1.41E-03	NO
18718	CG8359	1.31	2.73E-08	1.16E-05	NO
24351	CG14074	1.31	8.78E-08	1.95E-05	NO
27678	CG17490	1.31	3.26E-05	5.44E-04	NO
3828	mod(mdg4)	1.32	2.92E-04	2.66E-03	NO
16095	CG4730	1.32	3.85E-05	6.04E-04	NO
14217	Gem2	1.32	1.62E-04	1.70E-03	NO

2606	Irbp	1.32	1.08E-02	4.38E-02	NO
943	CG2051	1.33	1.16E-04	1.34E-03	NO
3069	Trf4-2	1.33	1.54E-04	1.64E-03	NO
16493	CG32043	1.33	1.25E-04	1.41E-03	NO
2273	CG32243	1.33	2.55E-04	2.40E-03	NO
8897	CR32027	1.34	1.36E-08	1.06E-05	NO
2911	Mal-B1	1.35	2.08E-03	1.21E-02	NO
839	alpha-Man-I	1.37	3.01E-03	1.61E-02	NO
21709	CG12717	1.38	8.20E-04	5.86E-03	NO
23521	ana3	1.38	8.04E-05	1.03E-03	NO
6051	CG7110	1.40	3.51E-09	7.08E-06	NO
28629	Alh	1.41	7.13E-05	9.43E-04	NO
515	cutlet	1.41	1.18E-02	4.72E-02	NO
29235	xmas-2	1.42	9.58E-04	6.61E-03	NO
8939	exu	1.43	1.52E-07	2.51E-05	NO
891	wapl	1.44	6.54E-03	2.95E-02	NO
4828	CG11307	1.45	6.20E-05	8.48E-04	NO
28385	CG13029	1.46	1.82E-05	3.62E-04	NO
3461	Caf1-105	1.46	3.47E-03	1.80E-02	NO
14024	pad	1.48	1.61E-05	3.33E-04	NO
6936	inx7	1.55	3.04E-08	1.26E-05	NO
13763	CG13749	1.58	1.60E-05	3.33E-04	NO
23013	Try29F	1.75	6.15E-04	4.74E-03	NO
2288	nos	1.78	1.08E-02	4.38E-02	NO
1570	jumu	1.95	6.40E-03	2.90E-02	NO
2473	Vm32E	-6.25	1.96E-06	8.92E-05	YES
30302	Vml	-6.21	2.70E-10	1.74E-06	YES
4113	Vm34Ca	-5.03	5.65E-07	4.63E-05	YES
11455	Vm34Ca	-4.95	1.64E-08	1.10E-05	YES
24855	Vm26Ac	-4.77	7.04E-09	8.73E-06	YES
21669	1-Dec	-4.72	6.65E-08	1.78E-05	YES
3366	Vm26Aa	-4.49	2.83E-06	1.12E-04	YES
17378	psd	-4.43	1.85E-09	5.41E-06	YES
29032	CG17192	-4.28	4.01E-11	6.47E-07	YES
2304	CG17192	-4.17	7.54E-08	1.85E-05	YES
16599	CG12398	-4.14	9.00E-09	9.06E-06	YES
20206	CG32751	-4.10	3.41E-11	6.47E-07	YES
8521	Vm34Ca	-4.03	1.96E-08	1.10E-05	YES
7692	Vm26Ab	-3.94	1.55E-08	1.09E-05	YES
19212	CG31775	-3.92	2.28E-06	9.83E-05	YES
8288	SNCF	-3.86	1.05E-04	1.25E-03	YES
19449	halo	-3.81	1.70E-04	1.77E-03	YES
9916	CG4440	-3.76	3.01E-04	2.71E-03	YES
9509	Jon25Bi	-3.76	1.87E-06	8.75E-05	YES
6430	CG10035	-3.59	1.31E-03	8.44E-03	YES
2855	CG13427	-3.57	3.61E-04	3.12E-03	YES
30160	CG17738	-3.57	8.61E-09	9.06E-06	YES
17567	Tom	-3.52	5.22E-04	4.17E-03	YES
31495	CG13427	-3.47	2.40E-04	2.28E-03	YES
19132	CG4830	-3.47	8.06E-08	1.85E-05	YES
13587	fit	-3.43	3.55E-07	3.70E-05	YES
17996	CG11912	-3.41	2.03E-08	1.10E-05	YES
29719	psd	-3.34	1.40E-09	4.60E-06	YES
12326	CG14915	-3.24	7.80E-04	5.65E-03	YES
11878	CG6704	-3.23	4.98E-07	4.32E-05	YES
14556	Pcp	-3.21	2.61E-08	1.15E-05	YES
7219	CG12057	-3.12	9.20E-08	1.95E-05	YES
4917	CG13215	-3.07	1.38E-05	3.03E-04	YES
28088	Fcp3C	-3.06	1.78E-03	1.07E-02	YES
25042	Bro	-3.05	4.84E-06	1.53E-04	YES
19951	Bsg25A	-3.03	1.49E-04	1.61E-03	YES
10448	BobA	-2.96	2.73E-04	2.53E-03	YES
8496	CG31259	-2.94	9.23E-06	2.32E-04	YES
15107	CG10592	-2.91	6.86E-10	3.16E-06	YES
21893	CG15634	-2.87	2.18E-04	2.12E-03	YES
19743	CG5150	-2.79	1.76E-08	1.10E-05	YES
10286	Cht9	-2.77	4.71E-08	1.55E-05	YES
371	CG14317	-2.74	2.93E-04	2.66E-03	YES
16597	vanin-like	-2.71	1.54E-05	3.23E-04	YES
26487	Fcp3C	-2.70	2.96E-03	1.60E-02	YES

28914	CG34137	-2.69	1.37E-04	1.51E-03	YES
27036	CG31041	-2.69	4.54E-06	1.47E-04	YES
13908	Pebp1	-2.69	1.06E-06	6.47E-05	YES
12859	Z600	-2.68	1.55E-03	9.59E-03	YES
24758	Pebp1	-2.68	1.36E-06	7.31E-05	YES
28439	CG11911	-2.67	1.50E-08	1.09E-05	YES
2525	CG5326	-2.62	2.71E-05	4.76E-04	YES
3727	scw	-2.61	2.21E-04	2.14E-03	YES
27625	CG8960	-2.53	9.53E-05	1.17E-03	YES
28142	CG4563	-2.52	1.77E-07	2.64E-05	YES
8739	CG8960	-2.51	1.18E-04	1.36E-03	YES
6583	Jon99Fi	-2.49	8.00E-07	5.51E-05	YES
20332	CG18180	-2.49	1.44E-06	7.59E-05	YES
6613	ndl	-2.48	2.37E-10	1.74E-06	YES
16457	wbl	-2.44	2.94E-07	3.37E-05	YES
27928	CG31041	-2.43	1.14E-05	2.66E-04	YES
9690	CG14014	-2.41	1.01E-04	1.21E-03	YES
27466	CG33109	-2.40	4.05E-09	7.21E-06	YES
4532	sala	-2.37	1.84E-03	1.10E-02	YES
14899	CG15876	-2.35	3.17E-06	1.19E-04	YES
8011	CG33109	-2.35	1.85E-08	1.10E-05	YES
18569	BobA	-2.28	8.82E-04	6.21E-03	YES
25036	CG13215	-2.27	1.44E-05	3.11E-04	YES
17505	CR43264	-2.25	4.61E-06	1.49E-04	YES
14062	CG9468	-2.22	7.79E-08	1.85E-05	YES
27584	CG31259	-2.18	2.65E-05	4.67E-04	YES
12091	Uro	-2.14	4.54E-05	6.79E-04	YES
18324	CG11741	-2.14	2.44E-03	1.37E-02	YES
7149	CG14187	-2.14	7.76E-03	3.38E-02	YES
16073	CG13998	-2.12	1.18E-06	6.82E-05	YES
24424	CG13992	-2.10	5.04E-08	1.60E-05	YES
26792	CG14500	-2.09	2.21E-06	9.62E-05	YES
26563	Jon66Ci	-2.08	1.75E-05	3.53E-04	YES
7900	Jon44E	-2.06	2.91E-06	1.14E-04	YES
17541	Ir7c	-2.04	2.34E-07	2.91E-05	YES
9676	CG13465	-2.03	1.86E-03	1.11E-02	YES
13826	CG34214	-2.03	7.66E-04	5.57E-03	YES
6055	Jon66Cii	-2.02	1.26E-07	2.32E-05	YES
2744	CG17571	-2.01	3.19E-06	1.19E-04	YES
16786	zetaTry	-2.00	1.05E-06	6.47E-05	YES
5951	CG9825	-1.99	1.42E-05	3.08E-04	YES
2530	CG13641	-1.97	1.55E-05	3.25E-04	YES
27775	CG13641	-1.97	5.99E-05	8.29E-04	YES
8558	CG9465	-1.96	1.08E-05	2.59E-04	YES
11013	CG14377	-1.94	3.94E-07	3.93E-05	YES
11918	Rtnl1	-1.94	1.14E-08	9.95E-06	YES
22903	CG13427	-1.94	3.90E-03	1.96E-02	YES
31343	Smvt	-1.94	1.17E-05	2.73E-04	YES
14469	CG8773	-1.94	2.03E-07	2.75E-05	YES
16375	CG10182	-1.93	6.92E-04	5.15E-03	YES
22199	zetaTry	-1.93	2.56E-06	1.06E-04	YES
23210	gammaTry	-1.92	1.41E-07	2.47E-05	YES
30074	CG10910	-1.91	1.22E-04	1.39E-03	YES
1217	CG9466	-1.90	7.02E-07	5.16E-05	YES
12983	CG30272	-1.89	5.60E-05	7.92E-04	YES
24430	Ela	-1.89	2.65E-08	1.15E-05	YES
10666	m1	-1.89	7.63E-07	5.38E-05	YES
914	nullo	-1.88	6.22E-07	4.87E-05	YES
24214	epsilonTry	-1.87	1.07E-06	6.47E-05	YES
11617	epsilonTry	-1.85	7.50E-07	5.32E-05	YES
12009	Ela	-1.84	7.98E-08	1.85E-05	YES
9990	deltaTry	-1.84	1.96E-07	2.73E-05	YES
24559	CG10407	-1.83	4.32E-04	3.60E-03	YES
18635	m4	-1.82	1.49E-04	1.61E-03	YES
21752	CG13042	-1.82	4.16E-06	1.39E-04	YES
24951	CG7025	-1.82	4.96E-07	4.32E-05	YES
18266	CG7631	-1.81	1.04E-07	2.04E-05	YES
25550	CG7300	-1.81	1.95E-08	1.10E-05	YES
7573	CR40597	-1.80	1.00E-07	2.02E-05	YES
28517	Npc2d	-1.80	1.70E-07	2.62E-05	YES

13937	CG7631	-1.80	9.06E-08	1.95E-05	YES
25010	CG10725	-1.80	2.45E-07	2.97E-05	YES
5848	CG16959	-1.80	3.21E-07	3.55E-05	YES
14288	CG31086	-1.79	1.75E-05	3.53E-04	YES
4008	Npc2e	-1.79	3.50E-06	1.26E-04	YES
9109	Jon65Aii	-1.79	6.68E-07	5.06E-05	YES
2920	CG31288	-1.78	3.96E-05	6.18E-04	YES
8243	CG13333	-1.77	8.61E-03	3.67E-02	YES
24212	Npc2d	-1.77	5.28E-08	1.62E-05	YES
15335	bond	-1.77	5.88E-08	1.68E-05	YES
16274	CG34224	-1.76	4.74E-03	2.28E-02	YES
5977	CG7912	-1.75	8.42E-05	1.06E-03	YES
7747	CG31233	-1.74	3.25E-06	1.21E-04	YES
8195	CG8562	-1.74	8.05E-08	1.85E-05	YES
1378	yellow-k	-1.74	7.12E-05	9.42E-04	YES
23563	CG7675	-1.74	2.11E-06	9.35E-05	YES
6126	Peritrophin-15a	-1.74	9.04E-06	2.29E-04	YES
12234	CG10725	-1.74	1.78E-06	8.58E-05	YES
15930	CG8560	-1.74	3.53E-07	3.70E-05	YES
29458	CG34205	-1.74	9.17E-07	6.02E-05	YES
26709	CG7912	-1.74	6.05E-05	8.34E-04	YES
21190	CG34266	-1.73	1.50E-04	1.61E-03	YES
16149	Atet	-1.72	7.66E-09	9.06E-06	YES
13901	Prx2540-2	-1.72	9.40E-06	2.35E-04	YES
13157	CG31869	-1.72	4.03E-07	3.96E-05	YES
21510	spo	-1.71	3.44E-06	1.24E-04	YES
12099	tj	-1.71	1.43E-09	4.60E-06	YES
4479	CG9837	-1.70	5.51E-06	1.65E-04	YES
2063	sug	-1.70	2.12E-05	4.02E-04	YES
5380	CG3819	-1.70	2.02E-06	9.10E-05	YES
3212	CG6733	-1.69	4.05E-08	1.45E-05	YES
13492	CG3348	-1.69	4.42E-05	6.68E-04	YES
18252	Ag5r2	-1.68	3.09E-06	1.17E-04	YES
29670	CG8834	-1.67	3.03E-05	5.17E-04	YES
28285	CG8785	-1.67	4.21E-06	1.41E-04	YES
897	CG7025	-1.67	1.13E-05	2.66E-04	YES
16691	CG10469	-1.66	6.10E-07	4.85E-05	YES
18261	CG34247	-1.66	3.85E-07	3.87E-05	YES
21883	CG18265	-1.64	4.87E-05	7.14E-04	YES
21135	CG8774	-1.63	1.45E-06	7.62E-05	YES
23119	CG13813	-1.63	3.12E-04	2.79E-03	YES
21463	CG31324	-1.62	1.09E-04	1.28E-03	YES
1420	CG14120	-1.61	1.94E-06	8.88E-05	YES
31666	CG4835	-1.61	2.12E-08	1.10E-05	YES
22610	CG8303	-1.61	7.74E-08	1.85E-05	YES
16800	CG14427	-1.59	6.40E-04	4.88E-03	YES
18829	Jon25Bii	-1.59	2.48E-07	2.99E-05	YES
9817	CG12374	-1.59	4.34E-07	4.13E-05	YES
3380	Jon74E	-1.59	3.33E-05	5.53E-04	YES
14829	CG14191	-1.59	2.50E-05	4.53E-04	YES
24618	CG6295	-1.58	6.77E-07	5.07E-05	YES
28544	CG14949	-1.58	8.16E-09	9.06E-06	YES
2654	CG16743	-1.57	7.15E-07	5.22E-05	YES
19991	CG15673	-1.56	3.53E-06	1.26E-04	YES
24018	CG13051	-1.56	7.03E-06	1.93E-04	YES
18835	CG30265	-1.56	8.73E-06	2.24E-04	YES
20818	CG6885	-1.55	7.40E-04	5.42E-03	YES
14424	PGRP-SC1b	-1.55	1.08E-03	7.27E-03	YES
20557	CG2930	-1.55	1.22E-06	6.90E-05	YES
19523	CG6738	-1.55	1.33E-06	7.23E-05	YES
31845	CG17571	-1.54	2.00E-07	2.73E-05	YES
4013	CG17633	-1.54	9.05E-06	2.29E-04	YES
22038	srw	-1.54	4.70E-05	6.96E-04	YES
27011	CG34367	-1.54	1.33E-05	2.95E-04	YES
28218	CG18269	-1.53	9.31E-06	2.33E-04	YES
9669	Hr46	-1.53	5.84E-08	1.68E-05	YES
13778	CG33306	-1.53	3.06E-06	1.17E-04	YES
5812	nimB3	-1.53	4.28E-06	1.42E-04	YES
20426	CG4020	-1.52	4.73E-09	7.26E-06	YES
23590	CG15282	-1.52	1.42E-03	8.98E-03	YES

17224	nimB3	-1.52	1.32E-05	2.95E-04	YES
17558	CG17633	-1.51	1.43E-05	3.10E-04	YES
5056	jhamt	-1.51	6.82E-06	1.91E-04	YES
28300	CG8952	-1.50	4.53E-08	1.52E-05	YES
2835	Smvt	-1.50	7.17E-05	9.47E-04	YES
28987	CG18258	-1.49	2.20E-07	2.87E-05	YES
5218	CG14624	-1.49	8.70E-06	2.24E-04	YES
32011	Jon25Biii	-1.49	7.06E-07	5.18E-05	YES
8235	fon	-1.48	1.08E-06	6.49E-05	YES
28743	CG17560	-1.48	8.46E-05	1.07E-03	YES
30393	Jon99Cii	-1.48	6.12E-05	8.40E-04	YES
7202	CG11796	-1.48	1.67E-05	3.41E-04	YES
3447	CG3344	-1.47	3.44E-05	5.64E-04	YES
8422	CG8628	-1.47	8.36E-06	2.19E-04	YES
28101	CG14095	-1.46	2.94E-06	1.14E-04	YES
26045	CG7443	-1.46	1.29E-05	2.90E-04	YES
6215	CG8952	-1.46	2.00E-07	2.73E-05	YES
10519	Cad99C	-1.46	9.58E-08	2.00E-05	YES
17777	CG18327	-1.46	5.59E-07	4.61E-05	YES
30272	CG18258	-1.46	1.31E-06	7.18E-05	YES
15652	CG5618	-1.45	1.44E-06	7.59E-05	YES
12226	CG4734	-1.45	8.04E-06	2.13E-04	YES
20430	CG31974	-1.44	6.94E-06	1.93E-04	YES
10347	LysS	-1.43	1.45E-03	9.11E-03	YES
1461	CG10469	-1.43	3.42E-05	5.62E-04	YES
22024	CG15531	-1.43	1.11E-06	6.60E-05	YES
21426	veil	-1.42	5.24E-08	1.62E-05	YES
11121	CG15828	-1.42	1.14E-07	2.15E-05	YES
6914	CG34329	-1.42	1.24E-07	2.31E-05	YES
27556	CG31681	-1.41	2.89E-06	1.13E-04	YES
17246	CG7381	-1.41	3.85E-06	1.33E-04	YES
4829	CG31974	-1.41	5.46E-06	1.65E-04	YES
17669	CG13813	-1.40	3.39E-04	2.97E-03	YES
3989	spo	-1.40	2.69E-04	2.50E-03	YES
12078	thetaTry	-1.40	5.12E-05	7.42E-04	YES
7932	CG18493	-1.40	5.37E-06	1.63E-04	YES
13676	TpnC47D	-1.39	1.63E-07	2.57E-05	YES
5928	Ilp4	-1.39	3.46E-03	1.79E-02	YES
13972	daw	-1.38	5.70E-07	4.65E-05	YES
15410	CG3290	-1.37	6.69E-05	8.99E-04	YES
5259	Jon99Fii	-1.37	1.19E-04	1.36E-03	YES
5247	CG6839	-1.36	3.19E-06	1.19E-04	YES
23767	TpnC73F	-1.35	1.55E-07	2.51E-05	YES
15385	CG6129	-1.35	3.62E-07	3.73E-05	YES
17236	CG31343	-1.35	7.65E-07	5.38E-05	YES
10623	CG3868	-1.34	1.50E-07	2.51E-05	YES
19488	CG13912	-1.34	1.29E-05	2.90E-04	YES
31671	CG5550	-1.34	1.98E-03	1.16E-02	YES
7667	Obp56a	-1.34	1.80E-04	1.84E-03	YES
4976	Listericin	-1.34	2.90E-06	1.13E-04	YES
28462	CG18327	-1.34	1.84E-06	8.71E-05	YES
16668	Obp49a	-1.34	1.09E-05	2.59E-04	YES
8377	inx3	-1.34	2.12E-08	1.10E-05	YES
21505	Jon65Aiii	-1.34	5.05E-06	1.58E-04	YES
10701	CG10096	-1.33	1.75E-06	8.49E-05	YES
4861	ftz	-1.32	3.12E-03	1.66E-02	YES
26783	CG42323	-1.32	3.07E-04	2.76E-03	YES
10474	CG31437	-1.32	1.17E-05	2.72E-04	YES
17776	CG1461	-1.32	2.74E-08	1.16E-05	YES
12164	CG4269	-1.31	6.83E-03	3.05E-02	YES
30887	TpnC47D	-1.31	1.89E-07	2.72E-05	YES
8149	CG9897	-1.31	2.42E-06	1.02E-04	YES
11979	CG15255	-1.31	7.18E-06	1.96E-04	YES
15800	CG32695	-1.30	3.10E-06	1.17E-04	YES
7862	CG5853	-1.30	3.80E-06	1.31E-04	YES
4676	CG5767	-1.30	7.07E-05	9.38E-04	YES
14746	sty	-1.30	7.35E-05	9.61E-04	YES
17196	CG6660	-1.30	3.08E-07	3.45E-05	YES
12852	CG4288	-1.29	4.40E-06	1.44E-04	YES
14106	CG42397	-1.29	1.38E-04	1.51E-03	YES

24069	CG13063	-1.29	6.88E-05	9.19E-04	YES
14909	CG6432	-1.29	1.42E-04	1.55E-03	YES
24409	CG42249	-1.27	3.43E-04	2.99E-03	YES
4662	CG31266	-1.27	1.84E-05	3.63E-04	YES
15196	CG17475	-1.27	1.03E-06	6.46E-05	YES
11177	CG13078	-1.27	1.50E-05	3.19E-04	YES
11209	eloF	-1.27	2.15E-07	2.83E-05	YES
27646	Amy-d	-1.27	3.72E-05	5.91E-04	YES
14971	LanA	-1.26	3.20E-08	1.29E-05	YES
10862	bwa	-1.26	4.52E-08	1.52E-05	YES
9650	mag	-1.26	1.30E-05	2.91E-04	YES
24795	CG13078	-1.26	9.60E-05	1.18E-03	YES
3354	nAcRbeta-21C	-1.26	6.97E-04	5.19E-03	YES
12399	Amy-p	-1.25	9.96E-05	1.21E-03	YES
24974	CrebA	-1.25	1.31E-06	7.16E-05	YES
14522	CG31266	-1.25	3.88E-05	6.08E-04	YES
31704	bwa	-1.25	5.08E-07	4.36E-05	YES
24009	CG8661	-1.25	6.62E-06	1.87E-04	YES
3884	CG12420	-1.25	3.56E-03	1.84E-02	YES
16124	CG17032	-1.24	3.04E-06	1.16E-04	YES
17682	CG15254	-1.24	6.82E-06	1.91E-04	YES
9595	CG10550	-1.24	2.20E-08	1.12E-05	YES
17977	CG9897	-1.24	7.06E-08	1.81E-05	YES
9423	CG7542	-1.24	1.29E-06	7.09E-05	YES
10977	CG5687	-1.23	1.42E-07	2.47E-05	YES
2079	CG15255	-1.23	1.63E-05	3.37E-04	YES
17989	Yp2	-1.23	8.93E-07	5.92E-05	YES
7060	CG8661	-1.23	2.40E-05	4.37E-04	YES
3141	CG4734	-1.23	4.83E-04	3.93E-03	YES
18016	CG31269	-1.23	2.44E-04	2.32E-03	YES
7558	br	-1.23	5.77E-05	8.07E-04	YES
3720	CG14762	-1.22	2.99E-04	2.70E-03	YES
31581	Kr-h1	-1.22	5.42E-04	4.29E-03	YES
12948	CG15254	-1.22	1.64E-05	3.37E-04	YES
29589	Obp99a	-1.22	9.23E-05	1.14E-03	YES
13633	CG31821	-1.22	3.41E-07	3.62E-05	YES
26853	Nep2	-1.22	1.97E-05	3.81E-04	YES
12561	CG33120	-1.21	7.59E-08	1.85E-05	YES
5495	CG17119	-1.21	1.84E-05	3.63E-04	YES
11446	CG4020	-1.21	9.51E-05	1.17E-03	YES
6493	Yp2	-1.21	2.97E-06	1.15E-04	YES
20155	CG15673	-1.20	1.78E-03	1.07E-02	YES
6856	Glut4EF	-1.19	1.52E-05	3.22E-04	YES
31223	Cpr65Ax1	-1.19	5.28E-03	2.48E-02	YES
16779	Glut4EF	-1.18	4.94E-06	1.55E-04	YES
3868	CG4653	-1.18	5.49E-05	7.81E-04	YES
5811	CG6660	-1.18	3.09E-07	3.45E-05	YES
28312	CG18179	-1.18	2.60E-05	4.63E-04	YES
27187	tal-AA	-1.17	1.05E-06	6.47E-05	YES
17614	CG31267	-1.16	1.80E-07	2.68E-05	YES
15618	kar	-1.16	3.57E-07	3.70E-05	YES
6151	CG17562	-1.16	7.32E-04	5.38E-03	YES
19121	amos	-1.16	5.34E-04	4.24E-03	YES
23621	CG13323	-1.15	6.85E-05	9.15E-04	YES
3730	CG8628	-1.15	7.00E-04	5.20E-03	YES
21042	CG33337	-1.14	1.28E-03	8.29E-03	YES
2419	CG14949	-1.13	3.48E-03	1.80E-02	YES
1825	v	-1.13	5.06E-06	1.58E-04	YES
7616	CG4783	-1.12	4.45E-07	4.19E-05	YES
6474	fon	-1.12	2.57E-08	1.15E-05	YES
19513	CG31267	-1.12	4.27E-07	4.10E-05	YES
15438	Orct2	-1.12	1.32E-04	1.46E-03	YES
27226	CG6776	-1.11	4.59E-05	6.85E-04	YES
3180	Jon65Aiv	-1.11	6.02E-04	4.66E-03	YES
6586	CG3759	-1.11	1.19E-06	6.82E-05	YES
22115	Orct	-1.11	2.59E-06	1.06E-04	YES
17924	CG17374	-1.10	7.67E-06	2.06E-04	YES
4232	PH4alphaEFB	-1.10	1.96E-06	8.92E-05	YES
17024	Ag5r	-1.10	5.22E-06	1.61E-04	YES
9452	Nrx-1	-1.09	1.94E-03	1.14E-02	YES

9988	LanB2	-1.09	1.25E-06	6.96E-05	YES
7032	CG13324	-1.08	3.95E-03	1.98E-02	YES
13370	CG13623	-1.08	3.70E-05	5.89E-04	YES
17853	cpo	-1.08	3.71E-07	3.75E-05	YES
27648	Obp83ef	-1.07	1.77E-06	8.58E-05	YES
4638	LysS	-1.07	2.83E-05	4.92E-04	YES
19304	Tig	-1.07	4.48E-07	4.19E-05	YES
22	Act87E	-1.07	2.82E-06	1.12E-04	YES
10797	CG9747	-1.07	2.93E-05	5.06E-04	YES
3128	CG13428	-1.05	2.51E-04	2.36E-03	YES
20281	CG3168	-1.04	6.16E-04	4.75E-03	YES
338	cher	-1.04	3.71E-03	1.89E-02	YES
8021	Rcd2	-1.03	4.20E-06	1.41E-04	YES
14684	Obp49a	-1.03	9.65E-03	4.02E-02	YES
29303	CrebA	-1.01	1.17E-06	6.77E-05	YES
15759	CG30047	-1.00	5.36E-07	4.51E-05	YES
8212	dgt3	1.00	4.09E-07	4.00E-05	YES
32138	Orc5	1.00	1.51E-06	7.81E-05	YES
2412	Mcm3	1.01	3.95E-03	1.98E-02	YES
12044	msb1l	1.01	3.83E-05	6.03E-04	YES
457	CG4617	1.01	9.32E-05	1.15E-03	YES
14113	CG2662	1.02	9.62E-07	6.20E-05	YES
2128	CG3430	1.03	1.23E-06	6.90E-05	YES
15239	CG14036	1.03	2.16E-07	2.83E-05	YES
27657	Cp110	1.03	7.59E-06	2.04E-04	YES
19500	CG12728	1.03	6.11E-06	1.78E-04	YES
5962	CG8152	1.04	1.91E-04	1.92E-03	YES
25586	CG9902	1.04	5.73E-07	4.65E-05	YES
18819	qkr54B	1.04	5.26E-06	1.61E-04	YES
22462	CG33156	1.05	1.98E-05	3.81E-04	YES
21441	CG12702	1.05	1.06E-04	1.25E-03	YES
25238	CG10336	1.05	1.13E-06	6.67E-05	YES
11914	CG12702	1.06	9.09E-05	1.13E-03	YES
16489	CG11329	1.06	2.49E-06	1.04E-04	YES
26842	mars	1.06	1.92E-07	2.73E-05	YES
445	sofe	1.06	5.79E-03	2.68E-02	YES
21320	CG15601	1.06	4.68E-07	4.29E-05	YES
29306	msl-3	1.06	1.75E-04	1.81E-03	YES
28375	fy	1.06	2.80E-07	3.26E-05	YES
28362	Sas-4	1.07	2.32E-07	2.91E-05	YES
18728	RnrL	1.07	5.86E-07	4.71E-05	YES
30961	E(var)3-9	1.07	1.94E-07	2.73E-05	YES
174	CG31109	1.07	1.32E-04	1.46E-03	YES
31243	mus301	1.07	1.04E-06	6.46E-05	YES
29932	Nek2	1.08	1.81E-06	8.64E-05	YES
15045	spn-A	1.08	3.17E-06	1.19E-04	YES
12264	dah	1.08	3.59E-06	1.27E-04	YES
7164	insv	1.09	1.65E-07	2.58E-05	YES
1630	CG15387	1.09	4.86E-03	2.33E-02	YES
28396	Oseg6	1.10	2.07E-08	1.10E-05	YES
19892	CR43670	1.10	2.35E-05	4.31E-04	YES
14319	Fancd2	1.10	3.68E-07	3.75E-05	YES
4859	Rad9	1.10	2.88E-05	4.98E-04	YES
2357	CG10050	1.10	1.44E-04	1.57E-03	YES
9812	msb1l	1.10	1.11E-06	6.60E-05	YES
15053	CG4854	1.11	1.73E-04	1.79E-03	YES
6698	CG8089	1.11	2.62E-08	1.15E-05	YES
8566	CG7101	1.11	1.69E-05	3.44E-04	YES
15051	CG33213	1.11	7.35E-06	1.99E-04	YES
1836	Klp67A	1.11	9.30E-03	3.90E-02	YES
13042	sas-6	1.12	6.65E-06	1.87E-04	YES
23470	tlk	1.12	1.81E-04	1.84E-03	YES
9659	dap	1.12	4.24E-06	1.41E-04	YES
29395	lola	1.12	2.37E-04	2.26E-03	YES
24160	CG42526	1.13	1.73E-07	2.62E-05	YES
26304	His3:CG31613	1.13	1.83E-04	1.86E-03	YES
11178	Su(var)2-10	1.13	3.08E-07	3.45E-05	YES
11954	tum	1.13	6.43E-08	1.76E-05	YES
12479	dnk	1.13	9.23E-06	2.32E-04	YES
8133	CoRest	1.13	6.77E-08	1.78E-05	YES

15253	CG4730	1.13	9.73E-09	9.22E-06	YES
25044	CG6136	1.13	2.33E-08	1.15E-05	YES
15137	CG8838	1.13	2.02E-05	3.86E-04	YES
6287	CG34406	1.13	1.14E-05	2.66E-04	YES
19886	CG14561	1.14	3.79E-06	1.31E-04	YES
14881	CG3457	1.14	8.51E-05	1.07E-03	YES
28268	Tsp96F	1.14	2.06E-06	9.25E-05	YES
22576	CG5245	1.14	2.29E-06	9.83E-05	YES
2170	CG14036	1.14	9.91E-05	1.20E-03	YES
24241	CG7386	1.14	5.51E-08	1.65E-05	YES
2160	Gen	1.14	9.36E-05	1.16E-03	YES
22910	Orc1	1.14	1.05E-06	6.47E-05	YES
30791	CG14561	1.15	1.02E-07	2.04E-05	YES
1573	CG9641	1.15	4.03E-04	3.40E-03	YES
15641	Orc1	1.15	7.89E-05	1.01E-03	YES
14194	CG13001	1.15	1.41E-07	2.47E-05	YES
28986	CG15047	1.15	1.96E-06	8.92E-05	YES
5978	CG5391	1.15	2.35E-05	4.32E-04	YES
562	CG12942	1.16	1.20E-02	4.77E-02	YES
1963	CG12717	1.16	6.24E-05	8.51E-04	YES
22534	Ctf4	1.16	9.14E-08	1.95E-05	YES
12397	nmdyn-D7	1.16	7.23E-06	1.97E-04	YES
189	DNApol-alpha60	1.16	1.85E-03	1.11E-02	YES
15785	IntS10	1.17	8.72E-09	9.06E-06	YES
29512	gammaTub37C	1.17	2.60E-06	1.07E-04	YES
27876	Orc2	1.17	2.05E-03	1.20E-02	YES
9011	sas-6	1.18	4.84E-06	1.53E-04	YES
16098	CG3032	1.18	8.21E-07	5.60E-05	YES
2239	CG33331	1.18	7.67E-08	1.85E-05	YES
31036	CG10638	1.18	1.78E-06	8.58E-05	YES
5897	CG32521	1.18	2.30E-06	9.88E-05	YES
1246	Sodh-1	1.18	1.92E-04	1.93E-03	YES
2213	CG8180	1.18	4.02E-03	2.01E-02	YES
14219	CG6683	1.18	3.42E-08	1.31E-05	YES
5157	CG33213	1.19	3.58E-07	3.70E-05	YES
12243	CG7386	1.19	9.91E-08	2.02E-05	YES
26806	His3:CG31613	1.19	2.01E-05	3.85E-04	YES
2221	sip2	1.19	5.28E-03	2.48E-02	YES
4685	CG3812	1.19	2.33E-07	2.91E-05	YES
8130	CG15436	1.19	1.48E-08	1.09E-05	YES
19929	CG3975	1.19	3.25E-08	1.29E-05	YES
2218	CG5359	1.20	1.69E-04	1.76E-03	YES
21471	CG31279	1.20	9.21E-08	1.95E-05	YES
13201	twe	1.20	1.55E-07	2.51E-05	YES
7528	Orc5	1.20	1.12E-06	6.60E-05	YES
8334	bora	1.20	5.81E-07	4.69E-05	YES
8188	CG31457	1.20	1.13E-07	2.15E-05	YES
211	CG32318	1.20	5.59E-08	1.65E-05	YES
18908	RhoGAP54D	1.20	1.47E-06	7.65E-05	YES
29104	CG42699	1.20	5.07E-07	4.35E-05	YES
27993	Msh6	1.22	1.19E-06	6.82E-05	YES
25552	CG10669	1.22	2.06E-08	1.10E-05	YES
7509	CG7130	1.22	3.57E-06	1.27E-04	YES
28230	CG3419	1.22	7.34E-05	9.60E-04	YES
31358	CR43670	1.22	5.73E-07	4.65E-05	YES
28568	CG10011	1.22	3.69E-05	5.89E-04	YES
13044	nmdyn-D6	1.23	5.89E-07	4.72E-05	YES
7435	Spindly	1.23	8.10E-08	1.85E-05	YES
30994	Sema-1a	1.23	1.73E-07	2.62E-05	YES
26328	Klp67A	1.23	5.06E-07	4.35E-05	YES
8186	Cks30A	1.23	1.00E-04	1.21E-03	YES
14104	trem	1.23	1.48E-05	3.17E-04	YES
23227	Orc2	1.23	4.98E-07	4.32E-05	YES
7242	CG6685	1.23	7.96E-08	1.85E-05	YES
12617	Rbf2	1.24	3.55E-06	1.27E-04	YES
7338	CG15643	1.24	5.90E-09	8.27E-06	YES
4534	nmdyn-D7	1.24	9.67E-08	2.00E-05	YES
6013	CG31251	1.24	4.19E-07	4.04E-05	YES
16164	Brf	1.25	1.58E-07	2.52E-05	YES
29664	Cks30A	1.25	1.66E-05	3.40E-04	YES

12144	Spc105R	1.25	7.40E-08	1.85E-05	YES
16796	Orc4	1.25	7.43E-07	5.32E-05	YES
7755	CycE	1.26	6.33E-07	4.92E-05	YES
8052	CG13690	1.26	1.89E-07	2.72E-05	YES
653	Msh6	1.27	5.77E-05	8.07E-04	YES
10059	ial	1.27	2.13E-06	9.40E-05	YES
20336	mei-S332	1.27	1.44E-07	2.48E-05	YES
7890	CG12713	1.27	4.59E-06	1.48E-04	YES
6483	rt	1.27	4.90E-07	4.32E-05	YES
3923	thr	1.27	8.98E-08	1.95E-05	YES
6047	CG1603	1.27	6.52E-07	5.00E-05	YES
15153	CG34398	1.28	3.74E-05	5.93E-04	YES
15795	CG4089	1.28	5.89E-08	1.68E-05	YES
20268	Rpn12R	1.28	2.18E-04	2.12E-03	YES
17136	mei-38	1.28	6.27E-08	1.73E-05	YES
22343	CG8247	1.29	5.12E-06	1.59E-04	YES
28436	CG31053	1.29	2.13E-06	9.40E-05	YES
9205	gd	1.29	6.04E-05	8.34E-04	YES
18423	CG2924	1.30	3.03E-07	3.44E-05	YES
27372	bcd	1.30	9.61E-05	1.18E-03	YES
9181	CG1603	1.30	1.76E-07	2.64E-05	YES
16972	CG6171	1.30	1.19E-07	2.24E-05	YES
31665	His1:CG33804	1.31	1.10E-05	2.60E-04	YES
20824	CG30096	1.31	1.46E-05	3.15E-04	YES
4805	CTPsyn	1.31	1.64E-07	2.57E-05	YES
23653	CG32364	1.31	4.36E-06	1.44E-04	YES
10353	CG11164	1.31	1.03E-05	2.49E-04	YES
2720	Chrac-16	1.31	1.93E-07	2.73E-05	YES
612	tef	1.31	1.37E-04	1.51E-03	YES
4421	l(2)dtl	1.32	3.27E-06	1.21E-04	YES
32161	CG31279	1.32	1.71E-07	2.62E-05	YES
15551	Klp67A	1.32	4.36E-07	4.13E-05	YES
8073	RnrS	1.32	2.18E-05	4.09E-04	YES
22705	snRNA:U1:82Eb	1.32	3.33E-05	5.53E-04	YES
27402	Chd3	1.32	4.43E-06	1.45E-04	YES
10314	Spindly	1.32	4.62E-08	1.54E-05	YES
13648	tobi	1.33	1.58E-06	8.01E-05	YES
2176	Spc25	1.33	9.57E-09	9.22E-06	YES
19854	CG34406	1.33	8.22E-08	1.87E-05	YES
10393	dnk	1.33	3.36E-06	1.23E-04	YES
3091	CG4570	1.34	9.51E-06	2.36E-04	YES
9208	CG11448	1.34	3.57E-08	1.34E-05	YES
7277	CG7130	1.34	6.13E-07	4.86E-05	YES
2899	CG7730	1.34	7.00E-08	1.81E-05	YES
26395	msd1	1.34	4.04E-06	1.37E-04	YES
2162	CG6928	1.34	7.86E-05	1.01E-03	YES
29997	mus101	1.34	4.93E-07	4.32E-05	YES
10537	CycE	1.34	1.26E-04	1.42E-03	YES
25867	cnir	1.35	1.28E-07	2.32E-05	YES
23347	Mis12	1.35	5.46E-07	4.55E-05	YES
21921	CG8786	1.36	6.75E-08	1.78E-05	YES
24962	CG32364	1.37	1.80E-06	8.63E-05	YES
18250	Hsp27	1.37	1.85E-07	2.69E-05	YES
5871	cid	1.37	1.45E-07	2.48E-05	YES
21358	CycE	1.37	6.09E-04	4.70E-03	YES
30374	Wnt5	1.38	2.90E-06	1.13E-04	YES
31710	Hsp27	1.38	4.94E-07	4.32E-05	YES
27963	Hsp27	1.39	4.85E-07	4.32E-05	YES
9733	scra	1.39	1.99E-08	1.10E-05	YES
23144	Hsp26	1.39	3.49E-07	3.67E-05	YES
32208	SAK	1.39	1.83E-07	2.69E-05	YES
20712	CG8247	1.40	1.55E-08	1.09E-05	YES
19672	CG14965	1.40	2.40E-07	2.93E-05	YES
1097	Chrac-16	1.41	1.51E-03	9.42E-03	YES
5241	Gen	1.41	1.47E-06	7.65E-05	YES
19210	spn-D	1.41	1.20E-05	2.75E-04	YES
6851	pim	1.42	1.87E-08	1.10E-05	YES
4320	His3:CG33830	1.43	1.57E-06	8.00E-05	YES
2094	CG30085	1.43	2.48E-03	1.39E-02	YES
5776	CG11360	1.43	1.84E-07	2.69E-05	YES

163	Hmr	1.44	1.33E-05	2.95E-04	YES
24177	CG5235	1.44	7.68E-07	5.39E-05	YES
4668	Poc1	1.44	3.30E-07	3.58E-05	YES
27801	CG14074	1.44	3.99E-07	3.94E-05	YES
30054	CG6752	1.44	3.38E-08	1.31E-05	YES
27207	CG18011	1.44	1.19E-08	1.01E-05	YES
19317	Klp61F	1.45	7.13E-07	5.22E-05	YES
26097	Hsp27	1.45	2.90E-07	3.35E-05	YES
26273	CG31807	1.46	7.41E-08	1.85E-05	YES
1654	CG6967	1.46	2.08E-05	3.96E-04	YES
3333	tef	1.46	5.35E-06	1.63E-04	YES
5763	Rpt3R	1.47	1.67E-05	3.41E-04	YES
3634	CG7650	1.48	3.78E-04	3.23E-03	YES
28336	CG31053	1.50	4.88E-08	1.59E-05	YES
11305	Orc2	1.50	6.31E-06	1.81E-04	YES
31303	His4:CG33905	1.50	3.48E-06	1.25E-04	YES
16959	neur	1.51	2.72E-06	1.10E-04	YES
22608	CG6425	1.51	3.29E-09	7.08E-06	YES
1969	CG11360	1.51	6.10E-08	1.71E-05	YES
10431	mei-218	1.51	8.27E-07	5.62E-05	YES
18333	CG31898	1.51	2.56E-09	6.33E-06	YES
17812	rt	1.52	2.02E-07	2.74E-05	YES
24828	CG17658	1.52	6.25E-09	8.40E-06	YES
27806	tobi	1.56	4.20E-08	1.46E-05	YES
15747	CG8526	1.57	2.49E-08	1.15E-05	YES
4170	CG13609	1.57	7.84E-10	3.16E-06	YES
29992	CG10445	1.57	2.53E-08	1.15E-05	YES
30962	CG2990	1.57	1.11E-07	2.15E-05	YES
20467	RhoGAP54D	1.57	1.32E-08	1.06E-05	YES
1321	sti	1.57	4.04E-04	3.41E-03	YES
17526	CG10445	1.59	2.53E-08	1.15E-05	YES
718	CG5245	1.59	2.38E-06	1.01E-04	YES
19122	CG12708	1.63	6.79E-08	1.78E-05	YES
10126	CG10013	1.63	1.39E-06	7.42E-05	YES
20602	Try29F	1.64	5.46E-04	4.31E-03	YES
4506	pon	1.64	9.40E-08	1.98E-05	YES
31954	CG32822	1.65	1.03E-08	9.50E-06	YES
29437	snRNA:U12:73B	1.72	5.17E-05	7.46E-04	YES
12094	CG14059	1.72	1.47E-07	2.49E-05	YES
4376	CG13609	1.75	2.58E-06	1.06E-04	YES
2384	CG6012	1.75	1.08E-05	2.58E-04	YES
3938	bam	1.82	2.62E-08	1.15E-05	YES
7070	Rad51D	1.84	5.48E-09	8.03E-06	YES
20415	mms4	1.88	1.39E-07	2.46E-05	YES
30607	CG14059	1.89	3.13E-06	1.18E-04	YES
25900	Ipod	1.89	6.64E-09	8.55E-06	YES
12221	Rad51D	1.92	8.19E-09	9.06E-06	YES
28010	snRNA:U11	2.21	1.21E-05	2.77E-04	YES
3615	Ilp7	2.65	2.48E-10	1.74E-06	YES
25153	CG15263	3.28	5.60E-07	4.61E-05	YES
26382	CG34040	3.32	5.02E-06	1.57E-04	YES
14890	CG13091	3.54	3.41E-09	7.08E-06	YES

Table B5: Log fold change in transcript abundance due to infection in virgin eggless females (Comparison A in Figure 5.1). This table contains all probes for genes significantly differentially expressed between virgin uninfected eggless females and virgin infected eggless females.

ProbeUID	Gene name (where available)	logFC (Uninfected virgin - Infected virgin)	p-value	B.H. Adj. p-value	Also significant in mated females?
7229	TotM	-1.80	9.68E-04	2.45E-02	NO
6289	CG30098	-1.29	1.16E-03	2.78E-02	NO
32174	TotA	-1.28	1.98E-03	4.03E-02	NO
29065	TotC	-1.21	1.84E-03	3.83E-02	NO
20513	Gp93	-1.17	7.81E-08	2.96E-05	NO
7493	CG2918	-1.15	6.21E-05	3.60E-03	NO
1120	CG8389	-1.12	2.65E-03	4.86E-02	NO
29360	CG17271	-1.11	9.59E-09	6.91E-06	NO
17787	CG16713	-1.09	4.81E-05	3.03E-03	NO
359	TRAM	-1.07	2.08E-07	6.10E-05	NO
21656	CG16713	-1.06	6.34E-05	3.66E-03	NO
13420	CG42816	-1.04	1.32E-06	2.25E-04	NO
10255	CG17271	-1.04	4.03E-06	5.09E-04	NO
17509	Ect3	-1.04	1.15E-04	5.64E-03	NO
7933	Idgf3	-1.03	2.64E-06	3.94E-04	NO
962	Mvl	-1.01	2.83E-06	4.08E-04	NO
3607	CG5493	-1.00	5.55E-04	1.65E-02	NO
3111	CG5999	1.01	2.17E-03	4.28E-02	NO
764	CG18673	1.03	1.35E-03	3.08E-02	NO
19108	CG42788	1.03	1.54E-04	6.93E-03	NO
13623	Tret1-1	1.04	1.05E-04	5.25E-03	NO
4178	CG1887	1.04	8.38E-04	2.19E-02	NO
7649	CG8112	1.07	4.95E-06	5.81E-04	NO
13500	Men	1.08	3.33E-07	8.46E-05	NO
24484	Men	1.09	2.09E-05	1.65E-03	NO
10821	CG8112	1.13	2.32E-06	3.59E-04	NO
4944	Men	1.15	3.37E-05	2.35E-03	NO
16971	fu12	1.16	4.56E-08	1.99E-05	NO
11673	CG10516	1.16	4.38E-04	1.41E-02	NO
5903	CG15199	1.19	1.71E-06	2.80E-04	NO
2320	Tret1-2	1.22	5.17E-05	3.20E-03	NO
16063	CG15199	1.25	5.52E-07	1.16E-04	NO
19603	Prat2	1.34	1.03E-05	9.77E-04	NO
25895	CG15120	1.41	1.62E-08	8.82E-06	NO
20602	Try29F	2.07	6.52E-05	3.70E-03	NO
23013	Try29F	2.09	3.62E-05	2.46E-03	NO
8032	CG11854	2.25	4.01E-05	2.65E-03	NO
27279	to	2.37	1.18E-04	5.73E-03	NO
31959	edin	-9.01	1.01E-10	3.60E-07	YES
29810	edin	-8.97	1.69E-10	4.94E-07	YES
18652	CecA1	-8.56	1.39E-08	8.08E-06	YES
4303	CecC	-8.28	1.74E-09	2.13E-06	YES
16814	CecA2	-8.28	2.64E-08	1.29E-05	YES
12179	CecA2	-8.24	2.32E-08	1.21E-05	YES
31592	CecC	-8.06	8.54E-10	1.38E-06	YES
4300	AttA	-7.01	3.19E-07	8.17E-05	YES
8070	AttA	-6.53	1.03E-06	1.88E-04	YES
22744	CG14322	-6.50	6.86E-10	1.30E-06	YES
27879	AttB	-6.37	4.01E-07	9.58E-05	YES
31794	AttA	-6.25	3.18E-07	8.17E-05	YES
11454	AttD	-6.23	4.67E-06	5.58E-04	YES
12774	CecB	-5.67	3.06E-07	8.07E-05	YES
23177	DptB	-5.52	1.29E-09	1.97E-06	YES
31396	DptB	-5.38	2.64E-09	2.78E-06	YES
1173	CecB	-5.04	1.80E-04	7.69E-03	YES
11140	Dpt	-4.98	3.69E-09	3.49E-06	YES
26123	PGRP-SB1	-4.94	3.51E-11	2.20E-07	YES
3715	CG10814	-4.81	6.11E-10	1.30E-06	YES
1240	Mtk	-4.72	7.61E-08	2.92E-05	YES
10953	PGRP-SB1	-4.21	5.71E-10	1.30E-06	YES
6382	CG4757	-4.12	1.52E-08	8.42E-06	YES
6203	CG2217	-4.10	4.09E-11	2.20E-07	YES

27837	IM18	-4.00	3.29E-08	1.49E-05	YES
971	AttC	-3.99	1.26E-07	4.37E-05	YES
20476	PGRP-LB	-3.84	1.37E-09	1.98E-06	YES
229	PGRP-SB2	-3.80	9.58E-11	3.60E-07	YES
19026	Ect3	-3.60	7.08E-09	5.55E-06	YES
13593	CG14190	-3.39	4.45E-10	1.10E-06	YES
19212	CG31775	-3.19	5.17E-08	2.17E-05	YES
7484	CG6361	-3.13	7.12E-09	5.55E-06	YES
16560	pirk	-3.03	4.05E-07	9.60E-05	YES
9914	pirk	-3.01	6.54E-07	1.32E-04	YES
31468	CG34054	-2.94	4.49E-07	1.01E-04	YES
14348	CG34054	-2.94	4.96E-07	1.09E-04	YES
13290	Dro	-2.90	8.54E-11	3.60E-07	YES
21150	Hsp70Aa	-2.88	8.96E-06	8.83E-04	YES
13809	Def	-2.87	1.53E-04	6.87E-03	YES
17460	CG30080	-2.86	9.76E-07	1.83E-04	YES
12003	CG13077	-2.86	3.92E-09	3.52E-06	YES
3885	Def	-2.83	4.86E-04	1.53E-02	YES
30684	CecA1	-2.83	7.87E-10	1.38E-06	YES
28824	CG13422	-2.82	2.74E-07	7.80E-05	YES
9600	eg	-2.81	2.68E-09	2.78E-06	YES
30225	IM23	-2.78	1.24E-04	5.92E-03	YES
5420	CG13905	-2.64	2.95E-06	4.18E-04	YES
9501	PGRP-LF	-2.61	2.18E-09	2.43E-06	YES
31207	PGRP-SD	-2.57	4.12E-07	9.66E-05	YES
12164	CG4269	-2.57	7.68E-06	8.17E-04	YES
20699	Drs-l	-2.56	3.01E-07	8.03E-05	YES
19926	spirit	-2.55	4.10E-06	5.13E-04	YES
17438	CG42559	-2.29	1.35E-12	4.35E-08	YES
14390	Fst	-2.19	1.12E-06	1.98E-04	YES
30759	Tsf1	-2.14	3.43E-07	8.62E-05	YES
31247	Tsf1	-2.12	5.12E-07	1.11E-04	YES
1474	CG11425	-2.11	6.08E-07	1.26E-04	YES
28059	Tsf1	-2.10	1.88E-07	5.80E-05	YES
16012	CG18563	-2.10	3.14E-07	8.16E-05	YES
8604	CG5527	-2.09	2.87E-08	1.36E-05	YES
3102	CG31775	-2.04	4.98E-07	1.09E-04	YES
14378	IM1	-2.00	9.52E-06	9.21E-04	YES
22102	Rel	-1.99	1.94E-07	5.80E-05	YES
325	PGRP-LC	-1.99	1.51E-08	8.42E-06	YES
17355	CG30026	-1.98	6.42E-08	2.55E-05	YES
14019	Rel	-1.96	1.11E-07	3.90E-05	YES
14970	CG15023	-1.96	1.38E-07	4.60E-05	YES
2068	Rel	-1.94	1.07E-07	3.81E-05	YES
14034	CG32284	-1.93	2.04E-04	8.30E-03	YES
16923	Gadd45	-1.92	1.08E-05	1.01E-03	YES
9973	CG9989	-1.91	4.26E-05	2.76E-03	YES
20957	Diedel	-1.90	2.09E-05	1.65E-03	YES
20275	CG4267	-1.88	9.82E-06	9.39E-04	YES
24057	PGRP-LB	-1.84	4.65E-06	5.58E-04	YES
3152	PGRP-LC	-1.84	1.22E-08	7.89E-06	YES
9698	CG34427	-1.83	6.69E-09	5.52E-06	YES
21011	CG43194	-1.82	2.31E-06	3.59E-04	YES
3592	PGRP-LC	-1.81	1.10E-08	7.52E-06	YES
4761	TepII	-1.80	4.51E-07	1.01E-04	YES
12091	Uro	-1.78	2.96E-07	8.03E-05	YES
6455	CG5791	-1.78	6.98E-05	3.91E-03	YES
17976	CG30109	-1.77	6.42E-09	5.44E-06	YES
3201	TepII	-1.76	3.45E-07	8.62E-05	YES
7970	Mvl	-1.75	4.48E-09	3.90E-06	YES
9312	PGRP-LF	-1.75	9.65E-09	6.91E-06	YES
22368	TrpA1	-1.74	5.19E-08	2.17E-05	YES
4250	CG42807	-1.72	4.53E-07	1.01E-04	YES
16565	CG9989	-1.70	3.23E-05	2.28E-03	YES
12111	Hsp70Bbb	-1.67	2.50E-05	1.85E-03	YES
27244	IM10	-1.67	2.41E-05	1.81E-03	YES
2374	Ugt37b1	-1.64	9.25E-07	1.75E-04	YES
3710	yin	-1.61	4.00E-06	5.09E-04	YES
11023	CG14322	-1.61	1.92E-10	5.16E-07	YES
6697	CG8449	-1.61	2.97E-09	2.99E-06	YES

22404	Cec2	-1.59	1.41E-09	1.98E-06	YES
7345	Dbp73D	-1.58	4.26E-08	1.88E-05	YES
18536	PGRP-SC2	-1.55	6.47E-05	3.68E-03	YES
5995	CG33470	-1.53	1.07E-05	1.00E-03	YES
5954	CG7017	-1.52	3.35E-06	4.50E-04	YES
25317	RhoGAP18B	-1.52	4.44E-06	5.45E-04	YES
31068	CG14529	-1.51	8.89E-09	6.66E-06	YES
16567	Cyp6w1	-1.50	1.48E-07	4.81E-05	YES
13445	PGRP-SC2	-1.47	1.91E-04	7.97E-03	YES
19061	PGRP-SA	-1.47	1.31E-07	4.40E-05	YES
12288	Rgk1	-1.47	8.28E-06	8.37E-04	YES
18732	PGRP-SA	-1.47	1.94E-07	5.80E-05	YES
7703	IM2	-1.45	2.19E-04	8.66E-03	YES
15100	Hsp70Bb	-1.45	1.77E-03	3.73E-02	YES
7036	IM4	-1.44	5.83E-05	3.43E-03	YES
14914	CG8046	-1.44	6.57E-07	1.32E-04	YES
6338	CG6639	-1.43	1.37E-04	6.35E-03	YES
20835	CG15065	-1.43	1.57E-05	1.35E-03	YES
23994	CG6361	-1.40	3.00E-06	4.21E-04	YES
27245	CG7442	-1.38	2.78E-07	7.80E-05	YES
728	Irc	-1.38	3.52E-06	4.68E-04	YES
16781	CG43691	-1.36	6.59E-10	1.30E-06	YES
11241	scpr-C	-1.36	1.33E-04	6.24E-03	YES
21878	CG6188	-1.36	4.69E-06	5.58E-04	YES
6079	CG9733	-1.35	3.45E-04	1.21E-02	YES
7859	Cyp4p3	-1.34	1.36E-06	2.31E-04	YES
20907	betaTub60D	-1.34	4.71E-06	5.58E-04	YES
18082	PGRP-LC	-1.34	4.17E-07	9.66E-05	YES
2333	CG6330	-1.33	1.57E-06	2.59E-04	YES
9756	IM3	-1.32	7.42E-05	4.09E-03	YES
5550	CG15550	-1.32	8.88E-07	1.69E-04	YES
7947	yellow-f	-1.32	1.08E-07	3.81E-05	YES
6549	CG34215	-1.31	4.01E-05	2.65E-03	YES
24839	CG5527	-1.31	2.46E-06	3.74E-04	YES
27320	CG30098	-1.30	7.61E-04	2.06E-02	YES
14542	CG12428	-1.27	7.30E-06	7.87E-04	YES
28194	LpR2	-1.25	7.50E-05	4.11E-03	YES
24453	nimB1	-1.25	3.31E-05	2.33E-03	YES
20148	CG14743	-1.25	8.22E-06	8.35E-04	YES
11763	CG6188	-1.23	1.34E-05	1.19E-03	YES
17627	CG5849	-1.22	1.02E-04	5.16E-03	YES
32037	LpR2	-1.22	3.51E-05	2.40E-03	YES
27396	CG12413	-1.20	4.19E-06	5.22E-04	YES
18663	Mvl	-1.20	3.13E-09	3.06E-06	YES
3744	CG3831	-1.19	1.09E-06	1.95E-04	YES
11104	CG14642	-1.18	3.93E-09	3.52E-06	YES
21376	Or22c	-1.17	3.70E-11	2.20E-07	YES
28353	LpR2	-1.17	1.33E-05	1.19E-03	YES
21230	CG8046	-1.13	1.13E-06	1.98E-04	YES
14849	Pu	-1.12	9.61E-04	2.44E-02	YES
14847	yellow-f	-1.12	9.52E-06	9.21E-04	YES
18999	l(1)G0020	-1.12	1.78E-09	2.13E-06	YES
17020	Drs	-1.12	6.98E-08	2.74E-05	YES
28877	CG17230	-1.11	2.84E-06	4.09E-04	YES
29493	CG16836	-1.10	5.79E-05	3.42E-03	YES
16291	Idgf3	-1.09	1.07E-06	1.93E-04	YES
3209	CG16712	-1.06	3.98E-08	1.78E-05	YES
26467	IM3	-1.02	5.93E-05	3.48E-03	YES
7261	SerT	-1.02	2.77E-06	4.08E-04	YES
12077	CG30109	-1.02	3.09E-08	1.42E-05	YES
7227	Iris	1.01	3.38E-05	2.35E-03	YES
19902	CG10924	1.05	5.22E-05	3.21E-03	YES
6542	CG43051	1.08	3.08E-07	8.07E-05	YES
7109	lectin-28C	1.17	1.52E-09	2.05E-06	YES
19476	alpha-Est2	1.17	1.41E-04	6.45E-03	YES
20454	Obp99a	1.20	3.34E-04	1.18E-02	YES
30532	Lsp1beta	1.21	1.90E-04	7.94E-03	YES
4005	CG32425	1.24	2.60E-04	9.86E-03	YES
14385	CG17124	1.27	1.48E-04	6.73E-03	YES
24835	CG42788	1.27	3.35E-06	4.50E-04	YES

29202	CG32425	1.33	3.06E-04	1.11E-02	YES
11674	lectin-28C	1.34	1.03E-08	7.18E-06	YES
9542	CG8539	1.42	9.65E-06	9.31E-04	YES
5200	Lsp2	1.43	5.59E-04	1.66E-02	YES
850	CG4950	1.46	9.57E-05	4.92E-03	YES
5321	CG42351	1.50	2.81E-06	4.08E-04	YES
4479	CG9837	1.57	1.68E-05	1.41E-03	YES
29589	Obp99a	1.57	2.07E-04	8.35E-03	YES
23029	CG1887	1.62	5.22E-05	3.21E-03	YES
13708	CG42351	1.69	2.36E-06	3.64E-04	YES
24620	CG34136	1.89	2.56E-07	7.38E-05	YES
8392	CG34136	2.01	1.30E-07	4.40E-05	YES
15638	CG8147	3.10	2.67E-08	1.29E-05	YES

Table B6: Log fold change in transcript abundance due to infection in mated eggless females (Comparison B in Figure 5.1). This table contains all probes for genes significantly differentially expressed between mated uninfected eggless females and mated infected eggless females.

ProbeUID	Gene name (where available)	logFC (Uninfected virgin - Infected virgin)	p-value	B.H. Adj. p-value	Also significant in virgin females?
26237	TrpA1	-6.47	5.74E-12	6.18E-08	NO
13048	CG34296	-1.73	8.48E-08	3.04E-05	NO
4375	Fst	-1.66	3.40E-05	2.83E-03	NO
22213	CG31775	-1.52	1.66E-06	2.96E-04	NO
21762	CG11313	-1.48	1.71E-07	5.39E-05	NO
31738	IM4	-1.27	9.00E-06	1.07E-03	NO
363	CG30281	-1.21	3.86E-04	1.55E-02	NO
31071	CG12111	-1.21	3.02E-05	2.56E-03	NO
26212	CG6553	-1.16	2.21E-10	5.67E-07	NO
27871	CG3505	-1.16	9.59E-07	1.96E-04	NO
12684	IM4	-1.15	6.92E-04	2.30E-02	NO
27476	CG6553	-1.08	4.82E-09	3.79E-06	NO
21357	Cyp4p3	-1.08	1.08E-05	1.21E-03	NO
20843	NUCB1	-1.08	9.37E-07	1.93E-04	NO
25423	CG16836	-1.07	3.39E-05	2.82E-03	NO
2482	CG18067	-1.05	8.53E-05	5.44E-03	NO
15375	CG13311	-1.05	4.37E-06	6.15E-04	NO
14854	CG11313	-1.04	7.27E-08	2.69E-05	NO
21084	CG13066	-1.04	1.43E-05	1.49E-03	NO
14505	NUCB1	-1.04	7.32E-05	4.82E-03	NO
14277	QC	-1.02	7.16E-04	2.35E-02	NO
22923	IM4	-1.02	6.17E-05	4.19E-03	NO
10796	CG14529	-1.02	1.11E-07	3.84E-05	NO
15297	CG8543	-1.01	2.68E-05	2.39E-03	NO
16766	Cyp309a2	-1.00	1.36E-06	2.56E-04	NO
10726	regucalcin	1.04	1.55E-07	4.99E-05	NO
30795	UGP	1.10	1.78E-04	9.10E-03	NO
8316	Obp83cd	1.12	3.07E-09	2.83E-06	NO
19132	CG4830	1.31	2.09E-04	1.00E-02	NO
16849	Lsp2	1.34	5.34E-04	1.94E-02	NO
29810	edin	-8.99	1.65E-10	4.82E-07	YES
31959	edin	-8.87	1.18E-10	4.77E-07	YES
18652	CecA1	-7.89	3.03E-08	1.41E-05	YES
4303	CecC	-7.87	2.86E-09	2.76E-06	YES
12179	CecA2	-7.68	4.55E-08	1.88E-05	YES
16814	CecA2	-7.67	5.49E-08	2.14E-05	YES
31592	CecC	-7.59	1.54E-09	2.00E-06	YES
22744	CG14322	-7.16	2.64E-10	5.67E-07	YES
4300	AttA	-6.78	4.38E-07	1.13E-04	YES
11454	AttD	-6.18	5.03E-06	6.84E-04	YES
8070	AttA	-6.11	1.87E-06	3.25E-04	YES
27879	AttB	-6.08	6.19E-07	1.44E-04	YES
31794	AttA	-5.94	5.18E-07	1.27E-04	YES
23177	DptB	-5.66	1.01E-09	1.47E-06	YES
12774	CecB	-5.57	3.63E-07	9.84E-05	YES
31396	DptB	-5.40	2.53E-09	2.61E-06	YES

11140	Dpt	-5.19	2.50E-09	2.61E-06	YES
1240	Mtk	-5.07	3.83E-08	1.69E-05	YES
27837	IM18	-4.98	3.89E-09	3.21E-06	YES
3715	CG10814	-4.86	5.54E-10	9.40E-07	YES
19212	CG31775	-4.65	1.34E-09	1.88E-06	YES
26123	PGRP-SB1	-4.55	8.00E-11	4.29E-07	YES
971	AttC	-4.44	4.61E-08	1.88E-05	YES
6203	CG2217	-4.32	2.41E-11	1.55E-07	YES
6382	CG4757	-4.23	1.17E-08	6.30E-06	YES
20476	PGRP-LB	-3.97	9.88E-10	1.47E-06	YES
10953	PGRP-SB1	-3.77	1.69E-09	2.09E-06	YES
1173	CecB	-3.70	1.72E-03	4.36E-02	YES
19026	Ect3	-3.66	5.98E-09	4.29E-06	YES
229	PGRP-SB2	-3.65	1.44E-10	4.77E-07	YES
13593	CG14190	-3.59	2.52E-10	5.67E-07	YES
13809	Def	-3.52	2.84E-05	2.48E-03	YES
3885	Def	-3.38	1.21E-04	7.08E-03	YES
30225	IM23	-3.23	3.62E-05	2.93E-03	YES
28824	CG13422	-3.18	8.71E-08	3.09E-05	YES
7484	CG6361	-3.15	6.62E-09	4.54E-06	YES
3102	CG31775	-3.10	9.00E-09	5.40E-06	YES
16560	pirk	-2.99	4.65E-07	1.17E-04	YES
9914	pirk	-2.95	8.08E-07	1.74E-04	YES
14348	CG34054	-2.90	5.62E-07	1.34E-04	YES
19926	spirit	-2.90	1.28E-06	2.44E-04	YES
14390	Fst	-2.88	8.37E-08	3.03E-05	YES
31468	CG34054	-2.86	5.74E-07	1.36E-04	YES
21150	Hsp70Aa	-2.83	1.06E-05	1.20E-03	YES
13290	Dro	-2.77	1.35E-10	4.77E-07	YES
9600	eg	-2.74	3.35E-09	3.00E-06	YES
12003	CG13077	-2.70	6.80E-09	4.56E-06	YES
20699	Drs-l	-2.68	1.89E-07	5.76E-05	YES
31207	PGRP-SD	-2.68	2.79E-07	7.88E-05	YES
9501	PGRP-LF	-2.56	2.59E-09	2.61E-06	YES
30684	CecA1	-2.53	2.29E-09	2.55E-06	YES
12164	CG4269	-2.49	9.86E-06	1.15E-03	YES
17438	CG42559	-2.44	7.20E-13	2.32E-08	YES
28059	Tsf1	-2.44	4.51E-08	1.88E-05	YES
31247	Tsf1	-2.44	1.36E-07	4.42E-05	YES
30759	Tsf1	-2.39	1.16E-07	3.96E-05	YES
17460	CG30080	-2.33	6.34E-06	8.01E-04	YES
22102	Rel	-2.29	5.21E-08	2.09E-05	YES
325	PGRP-LC	-2.26	4.35E-09	3.51E-06	YES
8604	CG5527	-2.26	1.37E-08	7.22E-06	YES
14378	IM1	-2.24	3.38E-06	5.11E-04	YES
16012	CG18563	-2.20	2.00E-07	5.98E-05	YES
2068	Rel	-2.19	3.26E-08	1.50E-05	YES
14019	Rel	-2.19	3.94E-08	1.72E-05	YES
6079	CG9733	-2.19	6.24E-06	7.98E-04	YES
5420	CG13905	-2.18	1.61E-05	1.64E-03	YES
14970	CG15023	-2.07	8.12E-08	2.97E-05	YES
20275	CG4267	-2.05	4.69E-06	6.49E-04	YES
9973	CG9989	-2.05	2.38E-05	2.20E-03	YES
4250	CG42807	-2.04	9.00E-08	3.15E-05	YES
24057	PGRP-LB	-2.02	1.98E-06	3.42E-04	YES
17355	CG30026	-2.02	5.26E-08	2.09E-05	YES
21878	CG6188	-2.00	1.24E-07	4.13E-05	YES
16923	Gadd45	-1.99	8.05E-06	9.79E-04	YES
20957	Diedel	-1.91	2.00E-05	1.94E-03	YES
16565	CG9989	-1.91	1.20E-05	1.30E-03	YES
11763	CG6188	-1.89	2.66E-07	7.74E-05	YES
6455	CG5791	-1.88	4.36E-05	3.29E-03	YES
3152	PGRP-LC	-1.86	1.08E-08	5.98E-06	YES
3592	PGRP-LC	-1.85	9.04E-09	5.40E-06	YES
9312	PGRP-LF	-1.83	5.93E-09	4.29E-06	YES
728	Irc	-1.81	2.87E-07	8.04E-05	YES
4761	TepII	-1.80	4.48E-07	1.14E-04	YES
9698	CG34427	-1.77	9.32E-09	5.46E-06	YES
3201	TepII	-1.74	3.80E-07	1.02E-04	YES
17976	CG30109	-1.74	7.77E-09	4.91E-06	YES

19061	PGRP-SA	-1.73	2.86E-08	1.36E-05	YES
24839	CG5527	-1.72	1.88E-07	5.76E-05	YES
18732	PGRP-SA	-1.72	4.19E-08	1.80E-05	YES
22368	TrpA1	-1.70	6.82E-08	2.58E-05	YES
7970	Mvl	-1.69	6.12E-09	4.29E-06	YES
12111	Hsp70Bbb	-1.68	2.39E-05	2.20E-03	YES
21011	CG43194	-1.65	5.52E-06	7.41E-04	YES
12288	Rgk1	-1.62	3.43E-06	5.16E-04	YES
16781	CG43691	-1.58	1.48E-10	4.77E-07	YES
20835	CG15065	-1.58	6.41E-06	8.04E-04	YES
3710	yin	-1.57	4.89E-06	6.73E-04	YES
22404	Cec2	-1.57	1.55E-09	2.00E-06	YES
11023	CG14322	-1.57	2.41E-10	5.67E-07	YES
23994	CG6361	-1.57	1.07E-06	2.15E-04	YES
11241	scpr-C	-1.57	4.18E-05	3.21E-03	YES
27244	IM10	-1.56	4.31E-05	3.27E-03	YES
5995	CG33470	-1.55	9.38E-06	1.11E-03	YES
1474	CG11425	-1.53	1.11E-05	1.23E-03	YES
2374	Ugt37b1	-1.52	1.85E-06	3.24E-04	YES
31068	CG14529	-1.49	1.01E-08	5.73E-06	YES
9756	IM3	-1.48	2.93E-05	2.52E-03	YES
7947	yellow-f	-1.48	3.59E-08	1.62E-05	YES
16567	Cyp6w1	-1.47	1.78E-07	5.51E-05	YES
7036	IM4	-1.46	5.30E-05	3.75E-03	YES
6338	CG6639	-1.44	1.26E-04	7.27E-03	YES
7859	Cyp4p3	-1.42	7.91E-07	1.72E-04	YES
15100	Hsp70Bb	-1.42	2.03E-03	4.84E-02	YES
6697	CG8449	-1.42	9.96E-09	5.73E-06	YES
14034	CG32284	-1.40	2.05E-03	4.86E-02	YES
25317	RhoGAP18B	-1.39	9.73E-06	1.14E-03	YES
28877	CG17230	-1.38	3.91E-07	1.04E-04	YES
27396	CG12413	-1.37	1.26E-06	2.42E-04	YES
7345	Dbp73D	-1.34	2.05E-07	6.05E-05	YES
2333	CG6330	-1.32	1.63E-06	2.96E-04	YES
6549	CG34215	-1.32	3.81E-05	2.99E-03	YES
5550	CG15550	-1.31	9.13E-07	1.91E-04	YES
7703	IM2	-1.31	4.76E-04	1.80E-02	YES
18536	PGRP-SC2	-1.31	2.50E-04	1.12E-02	YES
20907	betaTub60D	-1.26	8.27E-06	9.97E-04	YES
20148	CG14743	-1.26	7.48E-06	9.15E-04	YES
3744	CG3831	-1.26	6.57E-07	1.48E-04	YES
27245	CG7442	-1.25	6.94E-07	1.53E-04	YES
21376	Or22c	-1.25	1.98E-11	1.55E-07	YES
14847	yellow-f	-1.24	3.63E-06	5.41E-04	YES
24453	nimB1	-1.24	3.51E-05	2.88E-03	YES
18082	PGRP-LC	-1.23	9.35E-07	1.93E-04	YES
14542	CG12428	-1.22	1.10E-05	1.23E-03	YES
14914	CG8046	-1.21	3.26E-06	5.01E-04	YES
13445	PGRP-SC2	-1.21	8.56E-04	2.66E-02	YES
12091	Uro	-1.21	1.04E-05	1.19E-03	YES
18663	Mvl	-1.17	3.87E-09	3.21E-06	YES
17020	Drs	-1.17	4.40E-08	1.87E-05	YES
5954	CG7017	-1.17	3.49E-05	2.88E-03	YES
28353	LpR2	-1.15	1.44E-05	1.50E-03	YES
17627	CG5849	-1.15	1.64E-04	8.56E-03	YES
14849	Pu	-1.15	7.98E-04	2.53E-02	YES
21230	CG8046	-1.13	1.06E-06	2.14E-04	YES
27320	CG30098	-1.13	1.99E-03	4.78E-02	YES
29493	CG16836	-1.13	4.68E-05	3.43E-03	YES
3209	CG16712	-1.12	2.45E-08	1.18E-05	YES
11104	CG14642	-1.11	7.15E-09	4.67E-06	YES
26467	IM3	-1.11	3.06E-05	2.58E-03	YES
18999	l(1)G0020	-1.09	2.22E-09	2.55E-06	YES
12077	CG30109	-1.05	2.20E-08	1.09E-05	YES
16291	Idgf3	-1.01	2.12E-06	3.57E-04	YES
32037	LpR2	-1.01	1.64E-04	8.56E-03	YES
28194	LpR2	-1.01	4.07E-04	1.60E-02	YES
7261	SerT	-1.01	3.09E-06	4.80E-04	YES
24835	CG42788	1.03	2.12E-05	2.02E-03	YES
7227	Iris	1.05	2.43E-05	2.24E-03	YES

4005	CG32425	1.05	9.21E-04	2.80E-02	YES
20454	Obp99a	1.12	5.51E-04	1.98E-02	YES
29202	CG32425	1.13	1.02E-03	3.00E-02	YES
6542	CG43051	1.14	1.96E-07	5.90E-05	YES
19476	alpha-Est2	1.22	1.05E-04	6.37E-03	YES
14385	CG17124	1.23	1.86E-04	9.37E-03	YES
19902	CG10924	1.23	1.30E-05	1.39E-03	YES
850	CG4950	1.24	3.41E-04	1.42E-02	YES
5321	CG42351	1.25	1.36E-05	1.43E-03	YES
24620	CG34136	1.27	1.00E-05	1.16E-03	YES
13708	CG42351	1.28	2.78E-05	2.46E-03	YES
9542	CG8539	1.31	1.95E-05	1.91E-03	YES
4479	CG9837	1.34	6.19E-05	4.19E-03	YES
8392	CG34136	1.35	5.24E-06	7.07E-04	YES
7109	lectin-28C	1.35	3.82E-10	6.83E-07	YES
23029	CG1887	1.41	1.63E-04	8.56E-03	YES
30532	Lsp1beta	1.45	4.46E-05	3.32E-03	YES
11674	lectin-28C	1.49	3.80E-09	3.21E-06	YES
5200	Lsp2	1.69	1.55E-04	8.28E-03	YES
29589	Obp99a	1.75	8.68E-05	5.51E-03	YES
15638	CG8147	2.43	2.76E-07	7.88E-05	YES

Table B7: Log fold change in transcript abundance due to mating in uninfected eggless females (Comparison C in Figure 5.1). This table contains all probes for genes significantly differentially expressed between virgin uninfected eggless females and mated uninfected eggless females.

ProbeUID	Gene name (where available)	logFC (Uninfected virgin - Infected virgin)	p-value	B.H. Adj. p-value	Also significant in infected females?
19132	CG4830	-2.31	1.62E-06	2.26E-03	NO
29032	CG17192	-1.59	3.14E-05	1.01E-02	NO
8558	CG9465	-1.58	5.26E-05	1.27E-02	NO
13478	CG9463	-1.50	1.36E-05	6.72E-03	NO
11177	CG13078	-1.40	6.03E-07	1.78E-03	NO
17996	CG11912	-1.21	1.68E-08	5.41E-04	NO
689	ninaD	-1.19	3.96E-04	4.43E-02	NO
2654	CG16743	-1.15	6.59E-07	1.78E-03	NO
28517	Npc2d	-1.08	2.84E-06	3.05E-03	NO
24212	Npc2d	-1.07	3.89E-06	3.40E-03	NO
15107	CG10592	-1.06	1.50E-04	2.40E-02	NO
1217	CG9466	-1.04	1.40E-04	2.33E-02	NO
15777	tun	-1.01	9.97E-05	1.90E-02	NO
16063	CG15199	1.03	3.28E-06	3.16E-03	NO
2384	CG6012	1.04	2.41E-06	2.69E-03	NO
19603	Prat2	1.11	5.23E-05	1.27E-02	NO
13648	tobi	1.12	6.04E-05	1.40E-02	NO
363	CG30281	1.34	1.83E-04	2.76E-02	NO
9941	Mal-B1	1.57	9.45E-06	5.34E-03	NO
2911	Mal-B1	1.61	1.48E-05	6.95E-03	NO
14890	CG13091	1.87	4.67E-04	4.87E-02	NO
23013	Try29F	3.17	9.12E-07	1.84E-03	NO
20602	Try29F	3.20	1.40E-06	2.14E-03	NO
20206	CG32751	-1.89	6.89E-07	1.78E-03	YES
2304	CG17192	-1.42	2.01E-05	7.89E-03	YES
24795	CG13078	-1.35	1.70E-06	2.26E-03	YES
9509	Jon25Bi	-1.15	3.84E-05	1.08E-02	YES
25153	CG15263	1.59	4.04E-06	3.42E-03	YES
27661	Send1	1.69	1.75E-06	2.26E-03	YES
26382	CG34040	1.92	1.04E-07	1.02E-03	YES

Table B8: Log fold change in transcript abundance due to mating in infected eggless females (Comparison D in Figure 5.1). This table contains all probes for genes significantly differentially expressed between virgin infected eggless females and mated infected eggless females.

ProbeUID	Gene name (where available)	logFC (Uninfected virgin - Infected virgin)	p-value	B.H. Adj. p-value	Also significant in uninfected females?
26382	CG34040	1.58	6.61E-07	4.76E-03	NO
27661	Send1	1.79	1.07E-06	4.76E-03	NO
25153	CG15263	1.63	3.22E-06	1.04E-02	NO
20206	CG32751	-1.43	8.97E-06	2.15E-02	NO
2304	CG17192	-1.44	1.78E-05	2.86E-02	NO
24795	CG13078	-1.01	2.24E-05	3.39E-02	NO
9509	Jon25Bi	-1.21	2.42E-05	3.39E-02	NO
19212	CG31775	-1.64	2.32E-05	3.39E-02	YES

Table B9: Genes with a difference of 1.0 or more between virgin and mated females in the log fold change in transcript abundance after infection (Comparison A minus Comparison B from Figure 5.1). This table contains the difference between Comparison A and Comparison B and also the corresponding Comparison A and Comparison B values for each probe.

ProbeUID	Gene name	logFC: (vun-vinf)-(mun-minf)	Uncorrected p-value	logFC (vun-vinf)	p-value (vun-vinf)	B.H. adj. p-value (vun-vinf)	log FC (mun-minf)	p-value (mun-minf)	B.H. adj. p-value (mun-minf)
22213	CG31775	2.36	3.28E-07	-0.79	2.69E-04	2.42E-02	-3.15	5.94E-10	2.79E-06
17541	Ir7c	-1.47	1.06E-04	0.59	6.06E-03	2.06E-01	2.07	2.08E-07	1.40E-04
3102	CG31775	3.30	1.40E-04	-1.66	1.76E-03	9.30E-02	-4.97	1.45E-07	1.10E-04
20435	Tim17b2	1.01	1.91E-04	0.08	5.44E-01	8.76E-01	-0.93	2.05E-05	3.24E-03
15809	Dp1	1.12	3.41E-04	0.25	1.29E-01	6.80E-01	-0.87	1.62E-04	1.54E-02
12752	CG34232	-1.11	3.66E-04	-0.13	4.26E-01	8.25E-01	0.98	5.83E-05	7.09E-03
22773	Vha16-2	-1.42	4.09E-04	0.10	6.27E-01	9.06E-01	1.52	1.29E-05	2.29E-03
25497	HLHmgamma	-1.35	6.32E-04	-0.12	5.70E-01	8.87E-01	1.23	8.32E-05	9.39E-03
10474	CG31437	-1.14	6.74E-04	0.38	4.71E-02	5.65E-01	1.52	3.17E-06	8.11E-04
1474	CG11425	-1.37	1.04E-03	-2.59	2.00E-07	1.40E-04	-1.22	1.77E-04	1.64E-02
27714	CG15533	1.05	1.44E-03	-0.36	6.44E-02	6.06E-01	-1.41	7.99E-06	1.61E-03
27011	CG34367	-1.20	1.55E-03	0.43	5.67E-02	5.91E-01	1.63	7.92E-06	1.61E-03
27740	HLHmgamma	-1.14	1.58E-03	-0.22	2.83E-01	7.58E-01	0.93	5.93E-04	3.90E-02
21922	CG34278	-1.14	1.81E-03	0.18	3.67E-01	7.99E-01	1.32	3.85E-05	5.25E-03
6344	lectin-24A	-1.21	2.46E-03	-1.13	3.28E-04	2.77E-02	0.08	7.27E-01	9.71E-01
436	CG15553	1.28	2.58E-03	0.65	1.69E-02	3.75E-01	-0.63	2.05E-02	3.80E-01
27320	CG30098	-1.26	2.94E-03	-3.57	1.70E-08	2.29E-05	-2.31	1.19E-06	4.36E-04
5184	CG12607	1.01	3.13E-03	0.70	3.60E-03	1.48E-01	-0.31	1.25E-01	7.44E-01
13478	CG9463	1.69	3.15E-03	-0.28	3.97E-01	8.14E-01	-1.97	7.71E-05	8.87E-03
27279	to	1.33	4.25E-03	2.09	8.55E-06	1.78E-03	0.76	1.44E-02	3.17E-01
26629	Vml	1.52	4.29E-03	2.82	1.91E-06	5.92E-04	1.29	1.29E-03	6.57E-02
28953	CR40734	2.13	4.47E-03	0.78	9.00E-02	6.45E-01	-1.35	8.47E-03	2.30E-01
3731	Damm	-1.13	5.30E-03	0.29	2.26E-01	7.32E-01	1.43	8.06E-05	9.18E-03
13763	CG13749	-1.04	5.38E-03	-1.48	2.87E-05	4.41E-03	-0.44	6.09E-02	6.02E-01
19212	CG31775	2.06	5.69E-03	-3.30	1.10E-05	2.19E-03	-5.36	1.13E-07	9.08E-05
7692	Vm26Ab	1.22	6.15E-03	2.16	5.03E-06	1.25E-03	0.94	3.69E-03	1.37E-01
26866	Gel	1.02	6.65E-03	0.43	7.17E-02	6.17E-01	-0.59	1.90E-02	3.69E-01
4418	CG18273	1.09	7.03E-03	0.71	1.08E-02	2.91E-01	-0.37	1.32E-01	7.52E-01
21062	CG34291	1.12	7.14E-03	-0.20	4.15E-01	8.21E-01	-1.32	2.08E-04	1.80E-02
24554	HLHm5	-1.40	7.35E-03	-0.02	9.39E-01	9.90E-01	1.38	8.65E-04	4.98E-02
13587	fit	1.41	7.65E-03	1.84	1.01E-04	1.16E-02	0.43	1.85E-01	8.05E-01
4113	Vm34Ca	2.16	7.77E-03	2.98	6.50E-05	8.08E-03	0.82	1.07E-01	7.18E-01
6289	CG30098	-1.19	7.83E-03	-3.52	5.24E-08	5.44E-05	-2.34	2.72E-06	7.36E-04
16599	CG12398	1.13	9.29E-03	1.49	1.23E-04	1.37E-02	0.36	1.80E-01	8.02E-01
30302	Vml	1.19	9.50E-03	2.74	9.04E-07	3.42E-04	1.55	1.48E-04	1.46E-02
11455	Vm34Ca	1.42	1.01E-02	2.17	3.93E-05	5.52E-03	0.76	3.85E-02	5.01E-01
14784	CG13228	1.38	1.11E-02	0.81	2.81E-02	4.68E-01	-0.57	9.93E-02	7.03E-01
31207	PGRP-SD	1.11	1.18E-02	-2.21	4.92E-06	1.25E-03	-3.31	1.02E-07	8.44E-05
11170	CR9162	1.01	1.23E-02	0.74	9.98E-03	2.79E-01	-0.27	2.82E-01	8.62E-01
30532	Lsp1beta	1.08	1.26E-02	2.13	6.44E-06	1.45E-03	1.04	2.02E-03	8.94E-02
2374	Ugt37b1	1.07	1.27E-02	-1.16	8.77E-04	5.57E-02	-2.23	3.71E-06	9.14E-04
4135	CG43085	-1.17	1.52E-02	-2.10	2.08E-05	3.45E-03	-0.92	8.81E-03	2.35E-01
2911	Mal-B1	-1.30	1.84E-02	-0.61	9.15E-02	6.48E-01	0.69	6.22E-02	6.07E-01
21330	His2B:CG33868	1.29	1.86E-02	0.54	1.31E-01	6.80E-01	-0.76	4.31E-02	5.26E-01
17307	Jupiter	1.48	2.01E-02	1.00	2.53E-02	4.47E-01	-0.48	2.33E-01	8.33E-01
3257	CG14006	1.12	2.06E-02	0.46	1.41E-01	6.86E-01	-0.66	4.61E-02	5.42E-01
31247	Tsf1	1.24	2.08E-02	-1.60	5.21E-04	3.93E-02	-2.84	3.95E-06	9.44E-04
3201	TepII	-1.10	2.19E-02	-2.91	1.07E-06	3.82E-04	-1.82	7.49E-05	8.68E-03
16942	bt	1.14	2.32E-02	0.80	2.46E-02	4.43E-01	-0.35	2.81E-01	8.61E-01
27384	CG6244	-1.00	2.48E-02	-0.93	5.82E-03	2.01E-01	0.07	8.08E-01	9.84E-01
28289	Osi19	1.57	2.64E-02	-0.04	9.20E-01	9.85E-01	-1.61	3.50E-03	1.32E-01
18498	Npc2c	-1.54	2.68E-02	-0.02	9.54E-01	9.92E-01	1.52	4.67E-03	1.60E-01
18689	Ca-P60A	1.24	2.89E-02	0.93	2.18E-02	4.20E-01	-0.31	3.94E-01	9.04E-01
4330	Act5C	1.15	2.91E-02	0.59	9.45E-02	6.50E-01	-0.56	1.12E-01	7.25E-01
5625	CG6908	1.05	2.94E-02	0.19	5.42E-01	8.75E-01	-0.87	1.42E-02	3.15E-01
25486	CG10934	2.05	3.02E-02	0.33	5.82E-01	8.91E-01	-1.72	1.32E-02	3.04E-01
2251	CG17239	1.38	3.08E-02	0.31	4.48E-01	8.36E-01	-1.07	2.01E-02	3.78E-01
2446	CR32864	1.18	3.16E-02	0.57	1.18E-01	6.73E-01	-0.61	9.97E-02	7.03E-01
15516	ATPCL	1.05	3.32E-02	0.46	1.55E-01	6.99E-01	-0.58	8.04E-02	6.56E-01
4917	CG13215	1.37	3.52E-02	0.60	1.66E-01	7.04E-01	-0.77	8.09E-02	6.57E-01
18635	m4	-1.07	3.65E-02	0.20	5.39E-01	8.72E-01	1.26	2.23E-03	9.60E-02
2695	NT1	1.51	3.66E-02	0.81	9.89E-02	6.53E-01	-0.70	1.44E-01	7.65E-01

17986	CG14126	1.12	3.69E-02	0.61	9.29E-02	6.49E-01	-0.51	1.55E-01	7.79E-01
572	Hsp67Bc	-1.52	3.70E-02	-0.59	2.13E-01	7.26E-01	0.92	6.59E-02	6.18E-01
28133	CG9570	1.49	3.92E-02	0.98	5.21E-02	5.81E-01	-0.51	2.79E-01	8.60E-01
30759	Tsf1	1.12	4.06E-02	-1.61	7.01E-04	4.81E-02	-2.74	8.82E-06	1.73E-03
3331	Ehbp1	1.43	4.07E-02	0.74	1.18E-01	6.73E-01	-0.69	1.40E-01	7.60E-01
12761	CG42820	1.06	4.09E-02	0.75	4.08E-02	5.43E-01	-0.31	3.57E-01	8.94E-01
3789	Menl-1	1.02	4.54E-02	0.81	2.79E-02	4.67E-01	-0.21	5.23E-01	9.38E-01
2525	CG5326	1.17	4.69E-02	1.06	1.62E-02	3.68E-01	-0.12	7.52E-01	9.76E-01
29349	Cg25C	1.29	4.69E-02	0.86	5.76E-02	5.93E-01	-0.42	3.16E-01	8.81E-01
18942	r2d2	1.33	4.78E-02	1.10	2.41E-02	4.39E-01	-0.22	6.05E-01	9.53E-01
2890	CG13064	1.05	4.81E-02	0.83	3.08E-02	4.85E-01	-0.22	5.17E-01	9.37E-01
12684	IM4	1.09	4.88E-02	-0.09	7.99E-01	9.57E-01	-1.18	6.25E-03	1.90E-01
21456	CG33140	-1.02	4.98E-02	0.06	8.67E-01	9.73E-01	1.07	7.51E-03	2.15E-01
3411	CG31347	-1.01	5.09E-02	-0.39	2.52E-01	7.44E-01	0.62	8.46E-02	6.69E-01
17818	CG14515	1.33	5.10E-02	0.19	6.64E-01	9.19E-01	-1.14	2.27E-02	3.98E-01
3366	Vm26Aa	1.53	5.12E-02	2.19	1.12E-03	6.75E-02	0.66	2.08E-01	8.23E-01
16013	snoRNA:Psi18S-1854b	1.11	5.22E-02	0.71	7.67E-02	6.22E-01	-0.41	2.82E-01	8.62E-01
27411	yellow-d2	1.03	5.65E-02	0.77	4.48E-02	5.60E-01	-0.25	4.68E-01	9.26E-01
13817	rhea	1.12	5.67E-02	0.47	2.28E-01	7.32E-01	-0.65	1.10E-01	7.22E-01
31189	Ama	-1.06	5.73E-02	-0.21	5.53E-01	8.78E-01	0.84	3.59E-02	4.84E-01
377	CG13891	1.58	5.77E-02	0.67	2.27E-01	7.32E-01	-0.91	1.12E-01	7.26E-01
2027	Mur29B	1.07	5.80E-02	-0.51	1.76E-01	7.06E-01	-1.58	1.11E-03	5.95E-02
3771	CG43078	1.54	5.87E-02	0.77	1.65E-01	7.04E-01	-0.77	1.61E-01	7.86E-01
8864	Cyp4d14	1.22	5.89E-02	0.75	9.24E-02	6.48E-01	-0.46	2.77E-01	8.59E-01
7599	CG6954	1.14	5.91E-02	0.71	9.14E-02	6.47E-01	-0.43	2.81E-01	8.61E-01
21475	Doa	1.17	6.02E-02	0.81	6.41E-02	6.06E-01	-0.36	3.84E-01	9.00E-01
3773	S6k	2.37	6.03E-02	1.16	1.75E-01	7.05E-01	-1.21	1.57E-01	7.82E-01
2611	CG43896	1.27	6.06E-02	0.70	1.31E-01	6.80E-01	-0.57	2.10E-01	8.23E-01
9035	CG13325	1.02	6.33E-02	-0.26	4.62E-01	8.41E-01	-1.29	3.84E-03	1.39E-01
17112	dy	1.31	6.39E-02	0.67	1.67E-01	7.04E-01	-0.65	1.77E-01	7.99E-01
14390	Fst	-1.27	6.67E-02	-3.34	1.46E-05	2.70E-03	-2.07	7.33E-04	4.44E-02
6094	Cp7Fa	1.41	6.93E-02	1.13	4.31E-02	5.52E-01	-0.28	5.87E-01	9.49E-01
20832	CG34139	1.09	7.18E-02	0.49	2.33E-01	7.34E-01	-0.60	1.47E-01	7.67E-01
2286	CG11391	1.08	7.26E-02	0.76	7.38E-02	6.19E-01	-0.32	4.21E-01	9.12E-01
3191	nrm	1.10	7.33E-02	0.77	7.65E-02	6.22E-01	-0.33	4.14E-01	9.10E-01
5046	Met75Cb	1.65	7.46E-02	1.21	6.64E-02	6.08E-01	-0.44	4.69E-01	9.26E-01
27745	Cpr76Bc	1.02	7.55E-02	0.73	7.10E-02	6.16E-01	-0.29	4.52E-01	9.21E-01
9202	RNaseMRP:RNA	1.28	7.81E-02	0.56	2.57E-01	7.45E-01	-0.73	1.48E-01	7.68E-01
3109	ARY	1.12	8.05E-02	0.49	2.54E-01	7.44E-01	-0.62	1.56E-01	7.81E-01
18446	CG6628	1.08	8.55E-02	0.64	1.44E-01	6.92E-01	-0.45	2.93E-01	8.67E-01
3134	CG13526	1.80	8.59E-02	0.87	2.23E-01	7.30E-01	-0.93	1.94E-01	8.14E-01
6579	CG15642	1.08	8.69E-02	0.77	8.36E-02	6.39E-01	-0.31	4.64E-01	9.25E-01
2949	CG32655	3.44	8.70E-02	1.51	2.68E-01	7.51E-01	-1.94	1.63E-01	7.88E-01
6719	CG34430	1.11	8.86E-02	0.53	2.29E-01	7.32E-01	-0.57	1.97E-01	8.17E-01
2607	ChLD3	1.10	8.88E-02	0.66	1.39E-01	6.86E-01	-0.44	3.16E-01	8.81E-01
3342	Pgi	-1.42	9.05E-02	-0.52	3.57E-01	7.95E-01	0.90	1.24E-01	7.41E-01
2386	term	1.63	9.34E-02	0.98	1.45E-01	6.93E-01	-0.65	3.23E-01	8.83E-01
18896	Dscam4	1.51	9.37E-02	1.24	5.64E-02	5.91E-01	-0.27	6.53E-01	9.60E-01
2821	CG34375	2.08	9.55E-02	1.02	2.34E-01	7.35E-01	-1.07	2.12E-01	8.25E-01
3497	Lcp4	1.07	9.60E-02	0.68	1.31E-01	6.80E-01	-0.39	3.65E-01	8.97E-01
27494	CG7298	-1.16	9.70E-02	-1.01	4.76E-02	5.68E-01	0.15	7.44E-01	9.74E-01
3138	Ir7g	1.95	9.74E-02	0.97	2.27E-01	7.32E-01	-0.98	2.24E-01	8.28E-01
2614	CG13337	2.06	9.75E-02	0.68	4.12E-01	8.20E-01	-1.38	1.15E-01	7.32E-01
1261	CG17239	1.05	9.97E-02	-0.11	7.88E-01	9.53E-01	-1.17	1.72E-02	3.51E-01
25271	CG43367	1.03	1.00E-01	0.79	7.61E-02	6.22E-01	-0.23	5.74E-01	9.46E-01
3332	Ucp4C	1.66	1.01E-01	0.96	1.70E-01	7.04E-01	-0.70	3.07E-01	8.76E-01
3495	ymp	1.82	1.01E-01	1.16	1.35E-01	6.84E-01	-0.66	3.75E-01	8.98E-01
2618	CG17300	1.24	1.02E-01	0.65	2.15E-01	7.26E-01	-0.59	2.52E-01	8.46E-01
2627	CG32686	2.21	1.05E-01	1.01	2.77E-01	7.55E-01	-1.20	2.02E-01	8.21E-01
2790	CR43836	1.95	1.06E-01	0.97	2.40E-01	7.39E-01	-0.98	2.36E-01	8.35E-01
1214	GluRIIB	1.03	1.06E-01	0.22	6.05E-01	8.99E-01	-0.81	7.64E-02	6.44E-01
32190	CG13296	1.59	1.08E-01	1.20	8.88E-02	6.45E-01	-0.39	5.54E-01	9.43E-01
3492	CG1316	-1.29	1.08E-01	-0.26	6.21E-01	9.05E-01	1.02	7.48E-02	6.41E-01
710	Ptth	-1.17	1.08E-01	-0.61	2.28E-01	7.32E-01	0.57	2.55E-01	8.48E-01
3178	pUf68	1.59	1.10E-01	1.15	1.05E-01	6.60E-01	-0.45	5.02E-01	9.34E-01
2435	CG5521	1.00	1.10E-01	0.49	2.49E-01	7.43E-01	-0.51	2.38E-01	8.37E-01
9108	pip	1.17	1.10E-01	0.64	2.08E-01	7.23E-01	-0.54	2.85E-01	8.63E-01
2824	dpr13	3.00	1.10E-01	0.96	4.46E-01	8.35E-01	-2.04	1.24E-01	7.41E-01
15120	Cyp28c1	1.06	1.11E-01	0.85	7.28E-02	6.17E-01	-0.20	6.48E-01	9.58E-01
3200	CG32683	1.50	1.11E-01	1.01	1.26E-01	6.78E-01	-0.49	4.42E-01	9.20E-01

2836	CG43894	1.11	1.12E-01	0.69	1.59E-01	7.02E-01	-0.42	3.70E-01	8.98E-01
4739	CG9948	1.15	1.13E-01	1.03	5.23E-02	5.82E-01	-0.12	8.01E-01	9.84E-01
22784	CG40968	1.85	1.13E-01	1.47	8.05E-02	6.32E-01	-0.38	6.22E-01	9.56E-01
18569	BobA	-1.21	1.14E-01	-0.13	7.91E-01	9.54E-01	1.07	5.45E-02	5.82E-01
3205	CG1288	1.59	1.15E-01	0.90	1.98E-01	7.17E-01	-0.69	3.15E-01	8.81E-01
2998	Socs16D	2.17	1.16E-01	1.48	1.27E-01	6.79E-01	-0.69	4.60E-01	9.24E-01
3606	CG4683	1.31	1.17E-01	0.88	1.36E-01	6.84E-01	-0.44	4.39E-01	9.18E-01
11167	CG8568	1.12	1.17E-01	0.95	6.73E-02	6.10E-01	-0.17	7.15E-01	9.71E-01
3116	grh	1.38	1.18E-01	0.55	3.62E-01	7.97E-01	-0.84	1.76E-01	7.97E-01
3918	CG33233	1.41	1.19E-01	0.69	2.66E-01	7.50E-01	-0.72	2.46E-01	8.42E-01
2786	Kif3C	2.77	1.20E-01	0.99	4.12E-01	8.20E-01	-1.78	1.53E-01	7.76E-01
2457	cic	1.75	1.21E-01	0.63	4.07E-01	8.19E-01	-1.12	1.57E-01	7.82E-01
3390	pon	1.85	1.22E-01	0.82	3.15E-01	7.76E-01	-1.03	2.12E-01	8.25E-01
3484	CG3565	1.36	1.22E-01	0.78	1.98E-01	7.17E-01	-0.57	3.38E-01	8.91E-01
1372	Rbp1-like	1.23	1.22E-01	0.57	2.96E-01	7.65E-01	-0.66	2.29E-01	8.30E-01
6731	obst-A	1.40	1.23E-01	0.85	1.77E-01	7.06E-01	-0.55	3.74E-01	8.98E-01
3123	CG32971	1.56	1.23E-01	1.10	1.22E-01	6.74E-01	-0.45	5.03E-01	9.34E-01
1909	CG43689	1.19	1.23E-01	0.70	1.90E-01	7.11E-01	-0.49	3.54E-01	8.93E-01
3727	scw	1.12	1.24E-01	0.54	2.76E-01	7.55E-01	-0.58	2.49E-01	8.44E-01
2789	CG1273	1.78	1.24E-01	0.75	3.42E-01	7.90E-01	-1.03	2.00E-01	8.20E-01
3197	Uch-L3	-1.43	1.24E-01	-0.34	5.80E-01	8.90E-01	1.08	1.02E-01	7.08E-01
2825	CG14625	3.87	1.25E-01	1.61	3.48E-01	7.93E-01	-2.26	1.97E-01	8.16E-01
7333	chrb	1.10	1.26E-01	0.89	8.28E-02	6.38E-01	-0.20	6.72E-01	9.63E-01
3465	Ssdp	-1.25	1.26E-01	-0.18	7.38E-01	9.40E-01	1.06	7.13E-02	6.32E-01
1642	U2af38	-1.21	1.27E-01	-0.75	1.78E-01	7.06E-01	0.47	3.88E-01	9.02E-01
2798	CG10041	1.73	1.28E-01	0.70	3.66E-01	7.99E-01	-1.03	1.93E-01	8.13E-01
9676	CG13465	-1.15	1.29E-01	-0.15	7.60E-01	9.46E-01	0.99	6.99E-02	6.30E-01
2637	CG17127	2.16	1.29E-01	1.04	2.86E-01	7.60E-01	-1.12	2.55E-01	8.48E-01
558	EcR	-1.53	1.30E-01	-0.07	9.12E-01	9.84E-01	1.46	5.06E-02	5.63E-01
3185	HIP-R	-1.33	1.31E-01	-0.54	3.68E-01	8.00E-01	0.79	1.97E-01	8.16E-01
11454	AttD	-1.76	1.31E-01	-7.62	1.16E-06	4.10E-04	-5.86	1.31E-05	2.30E-03
3761	lea	2.02	1.33E-01	0.97	2.91E-01	7.62E-01	-1.05	2.59E-01	8.50E-01
19085	CG10249	1.30	1.33E-01	0.84	1.67E-01	7.04E-01	-0.46	4.31E-01	9.15E-01
2774	CG9235	1.35	1.33E-01	0.45	4.59E-01	8.40E-01	-0.90	1.55E-01	7.78E-01
7229	TotM	-1.36	1.35E-01	-4.69	1.07E-05	2.14E-03	-3.32	2.01E-04	1.76E-02
6296	CG17601	1.23	1.35E-01	0.92	1.16E-01	6.70E-01	-0.31	5.78E-01	9.47E-01
2772	Muc96D	2.18	1.36E-01	0.69	4.87E-01	8.50E-01	-1.49	1.47E-01	7.67E-01
3207	sphinx2	2.68	1.36E-01	1.33	2.81E-01	7.58E-01	-1.35	2.76E-01	8.59E-01
17567	Tom	-1.63	1.36E-01	0.04	9.61E-01	9.92E-01	1.66	4.08E-02	5.14E-01
3339	Cpr64Ac	1.98	1.39E-01	1.41	1.38E-01	6.84E-01	-0.58	5.24E-01	9.38E-01
2473	Vm32E	1.49	1.39E-01	3.78	1.62E-04	1.69E-02	2.29	5.60E-03	1.80E-01
13287	CG9875	1.78	1.40E-01	0.30	7.13E-01	9.33E-01	-1.48	8.83E-02	6.79E-01
3146	ppk20	1.11	1.41E-01	0.94	8.47E-02	6.40E-01	-0.17	7.37E-01	9.73E-01
3195	SKIP	2.07	1.42E-01	1.18	2.26E-01	7.32E-01	-0.88	3.59E-01	8.96E-01
1993	Ars2	1.22	1.43E-01	0.25	6.48E-01	9.13E-01	-0.96	1.05E-01	7.14E-01
3344	wac	-1.13	1.43E-01	-0.50	3.45E-01	7.92E-01	0.63	2.38E-01	8.37E-01
1371	Fas2	1.92	1.43E-01	0.73	4.11E-01	8.20E-01	-1.19	1.95E-01	8.14E-01
3076	blow	1.55	1.44E-01	0.92	2.13E-01	7.26E-01	-0.63	3.84E-01	9.01E-01
2020	clos	1.40	1.45E-01	1.09	1.12E-01	6.66E-01	-0.31	6.32E-01	9.56E-01
2888	CG31496	1.88	1.46E-01	1.08	2.29E-01	7.32E-01	-0.80	3.66E-01	8.97E-01
3600	nxf4	1.63	1.46E-01	1.17	1.42E-01	6.88E-01	-0.46	5.42E-01	9.41E-01
2613	CG15483	2.08	1.46E-01	1.33	1.84E-01	7.08E-01	-0.75	4.43E-01	9.20E-01
3174	Eig71Eg	1.88	1.48E-01	1.45	1.18E-01	6.73E-01	-0.43	6.23E-01	9.56E-01
2797	CG6118	4.09	1.48E-01	2.15	2.71E-01	7.52E-01	-1.94	3.19E-01	8.82E-01
2455	CG13727	1.09	1.51E-01	0.49	3.43E-01	7.91E-01	-0.59	2.57E-01	8.50E-01
2619	CG14434	1.43	1.53E-01	0.98	1.66E-01	7.04E-01	-0.45	5.03E-01	9.34E-01
2659	lbe	1.43	1.53E-01	0.37	5.83E-01	8.91E-01	-1.06	1.36E-01	7.55E-01
2775	gpp	2.14	1.53E-01	0.84	4.10E-01	8.20E-01	-1.30	2.15E-01	8.25E-01
2951	CG12679	1.52	1.54E-01	0.70	3.39E-01	7.88E-01	-0.82	2.66E-01	8.53E-01
3341	CG34292	1.16	1.54E-01	0.79	1.67E-01	7.04E-01	-0.37	5.08E-01	9.34E-01
2796	CG7560	2.67	1.55E-01	0.69	5.86E-01	8.92E-01	-1.98	1.39E-01	7.58E-01
2979	CG13023	1.62	1.57E-01	1.39	9.29E-02	6.49E-01	-0.23	7.63E-01	9.77E-01
3935	CG5715	1.02	1.57E-01	0.32	5.15E-01	8.62E-01	-0.70	1.67E-01	7.89E-01
2481	CG12684	1.55	1.57E-01	0.51	4.98E-01	8.55E-01	-1.05	1.76E-01	7.98E-01
2795	CG14253	3.43	1.57E-01	1.58	3.43E-01	7.90E-01	-1.85	2.72E-01	8.57E-01
2764	bft	1.24	1.58E-01	0.82	1.86E-01	7.10E-01	-0.42	4.78E-01	9.28E-01
3007	Ddr	1.74	1.58E-01	1.31	1.36E-01	6.84E-01	-0.43	6.04E-01	9.53E-01
734	myo	1.26	1.58E-01	0.19	7.56E-01	9.44E-01	-1.07	9.55E-02	6.92E-01
3318	Ipk1	1.60	1.60E-01	1.31	1.08E-01	6.63E-01	-0.28	7.11E-01	9.71E-01
2697	CG12027	1.24	1.61E-01	0.76	2.19E-01	7.28E-01	-0.48	4.27E-01	9.13E-01

3777	CG10038	-1.20	1.61E-01	-0.64	2.82E-01	7.58E-01	0.56	3.41E-01	8.91E-01
3478	CG12521	1.20	1.62E-01	0.87	1.54E-01	6.98E-01	-0.33	5.66E-01	9.45E-01
2165	CG6454	1.39	1.64E-01	0.79	2.54E-01	7.44E-01	-0.60	3.82E-01	9.00E-01
3224	Dscam4	1.05	1.64E-01	0.58	2.65E-01	7.50E-01	-0.47	3.69E-01	8.98E-01
2985	CG31047	1.49	1.64E-01	0.88	2.42E-01	7.40E-01	-0.62	4.01E-01	9.07E-01
1698	Lsp1alpha	1.34	1.64E-01	0.65	3.29E-01	7.82E-01	-0.69	2.99E-01	8.70E-01
3016	Poxm	1.66	1.66E-01	1.11	1.90E-01	7.10E-01	-0.56	4.96E-01	9.32E-01
2840	CG2025	1.52	1.67E-01	1.15	1.43E-01	6.91E-01	-0.37	6.16E-01	9.55E-01
13369	sti	1.06	1.68E-01	0.58	2.75E-01	7.54E-01	-0.48	3.66E-01	8.97E-01
3083	mthl8	1.28	1.68E-01	0.92	1.61E-01	7.02E-01	-0.36	5.72E-01	9.45E-01
3790	ATP7	-1.32	1.69E-01	-0.30	6.49E-01	9.13E-01	1.02	1.35E-01	7.55E-01
2460	CG14537	2.62	1.70E-01	1.29	3.27E-01	7.82E-01	-1.33	3.15E-01	8.81E-01
3011	CG9101	1.62	1.71E-01	1.32	1.19E-01	6.74E-01	-0.30	7.11E-01	9.71E-01
17108	CG12163	1.12	1.72E-01	0.62	2.76E-01	7.55E-01	-0.50	3.77E-01	8.98E-01
3791	Cam	-2.71	1.72E-01	-0.83	5.41E-01	8.74E-01	1.88	1.79E-01	8.00E-01
2280	CG15599	1.15	1.73E-01	0.63	2.80E-01	7.57E-01	-0.52	3.72E-01	8.98E-01
2870	ci	1.20	1.73E-01	1.09	8.93E-02	6.45E-01	-0.11	8.50E-01	9.89E-01
2712	otp	1.56	1.74E-01	0.93	2.45E-01	7.41E-01	-0.63	4.24E-01	9.12E-01
2603	robo	1.34	1.75E-01	0.47	4.91E-01	8.52E-01	-0.88	2.07E-01	8.22E-01
3967	m1	1.15	1.81E-01	1.03	1.00E-01	6.55E-01	-0.13	8.28E-01	9.87E-01
1267	CG9815	-1.20	1.86E-01	-1.03	1.16E-01	6.71E-01	0.17	7.79E-01	9.81E-01
2788	CG42329	1.33	1.87E-01	0.45	5.13E-01	8.61E-01	-0.88	2.15E-01	8.25E-01
2463	spz5	1.74	1.87E-01	0.77	3.97E-01	8.14E-01	-0.97	2.90E-01	8.65E-01
3067	CG17010	1.50	1.93E-01	1.11	1.77E-01	7.06E-01	-0.40	6.13E-01	9.54E-01
2865	CG8494	1.52	1.93E-01	0.78	3.33E-01	7.84E-01	-0.73	3.63E-01	8.97E-01
3260	CG8173	-1.11	1.98E-01	-0.89	1.50E-01	6.95E-01	0.22	7.05E-01	9.69E-01
1604	Lamp1	-1.38	1.98E-01	-1.21	1.19E-01	6.74E-01	0.17	8.12E-01	9.84E-01
2290	CG9865	1.48	2.00E-01	0.77	3.37E-01	7.86E-01	-0.71	3.75E-01	8.98E-01
2545	CG5172	1.30	2.03E-01	1.12	1.27E-01	6.79E-01	-0.18	7.97E-01	9.84E-01
1854	lt	1.02	2.05E-01	0.02	9.65E-01	9.93E-01	-1.00	9.01E-02	6.83E-01
3642	CG2924	-1.55	2.05E-01	-0.33	6.94E-01	9.29E-01	1.23	1.61E-01	7.86E-01
2711	Ace	1.37	2.05E-01	0.79	2.95E-01	7.65E-01	-0.58	4.36E-01	9.17E-01
3029	Met75Ca	1.21	2.05E-01	1.11	1.09E-01	6.64E-01	-0.10	8.77E-01	9.93E-01
3748	LS2	1.33	2.06E-01	1.05	1.62E-01	7.02E-01	-0.28	6.94E-01	9.68E-01
3541	CG12438	1.10	2.07E-01	0.82	1.85E-01	7.09E-01	-0.28	6.38E-01	9.56E-01
3506	CG18304	1.25	2.07E-01	1.34	6.67E-02	6.09E-01	0.10	8.86E-01	9.93E-01
2430	CG34173	1.09	2.08E-01	0.72	2.37E-01	7.37E-01	-0.37	5.33E-01	9.41E-01
2914	CG5342	1.23	2.08E-01	0.94	1.79E-01	7.06E-01	-0.30	6.58E-01	9.61E-01
2288	nos	-1.09	2.09E-01	-0.65	2.83E-01	7.58E-01	0.44	4.63E-01	9.25E-01
3392	CG10031	-1.35	2.09E-01	-0.97	2.00E-01	7.18E-01	0.37	6.10E-01	9.53E-01
2015	CG11148	2.70	2.09E-01	2.17	1.59E-01	7.02E-01	-0.53	7.15E-01	9.71E-01
1570	jumu	-1.08	2.10E-01	-0.79	1.97E-01	7.17E-01	0.29	6.20E-01	9.55E-01
262	ade5	1.07	2.11E-01	0.08	8.88E-01	9.79E-01	-0.99	1.11E-01	7.25E-01
2241	CG5367	-1.12	2.11E-01	-0.39	5.28E-01	8.68E-01	0.73	2.45E-01	8.42E-01
16129	CG3355	1.11	2.16E-01	0.78	2.16E-01	7.26E-01	-0.33	5.95E-01	9.51E-01
2321	CG32719	1.19	2.17E-01	0.79	2.49E-01	7.43E-01	-0.41	5.38E-01	9.41E-01
3049	CG31679	1.85	2.18E-01	1.47	1.69E-01	7.04E-01	-0.38	7.13E-01	9.71E-01
3963	Shab	1.99	2.18E-01	1.96	9.72E-02	6.53E-01	-0.03	9.75E-01	9.98E-01
2442	Cog7	1.60	2.19E-01	0.85	3.52E-01	7.93E-01	-0.76	4.02E-01	9.07E-01
2250	CG6945	-1.12	2.20E-01	-0.67	2.94E-01	7.65E-01	0.45	4.74E-01	9.27E-01
534	Sr-CIII	1.16	2.20E-01	0.13	8.41E-01	9.67E-01	-1.03	1.31E-01	7.51E-01
10448	BobA	-1.02	2.20E-01	0.10	8.56E-01	9.71E-01	1.12	6.86E-02	6.27E-01
3164	CG6983	1.50	2.22E-01	1.31	1.37E-01	6.84E-01	-0.18	8.26E-01	9.87E-01
15023	TfIIA-S	1.20	2.22E-01	1.22	8.97E-02	6.45E-01	0.02	9.72E-01	9.98E-01
1696	Cyp312a1	1.55	2.22E-01	1.15	2.01E-01	7.19E-01	-0.40	6.47E-01	9.58E-01
3263	n-syb	1.09	2.23E-01	0.55	3.73E-01	8.02E-01	-0.54	3.87E-01	9.02E-01
1838	CG34383	1.30	2.25E-01	0.60	4.15E-01	8.21E-01	-0.70	3.51E-01	8.93E-01
2640	CG2127	1.07	2.30E-01	0.69	2.70E-01	7.52E-01	-0.38	5.36E-01	9.41E-01
3298	CG10862	1.18	2.30E-01	0.83	2.34E-01	7.35E-01	-0.35	6.02E-01	9.53E-01
3422	Gr28b	1.03	2.32E-01	0.52	3.84E-01	8.08E-01	-0.51	3.94E-01	9.04E-01
9916	CG4440	-1.27	2.33E-01	-0.05	9.47E-01	9.90E-01	1.22	1.15E-01	7.31E-01
3347	mRpL23	1.06	2.35E-01	0.91	1.53E-01	6.97E-01	-0.14	8.12E-01	9.84E-01
3418	stv	1.46	2.37E-01	0.64	4.53E-01	8.38E-01	-0.82	3.42E-01	8.91E-01
2779	CG31687	1.27	2.39E-01	0.13	8.56E-01	9.71E-01	-1.14	1.44E-01	7.64E-01
2885	CG34167	1.20	2.39E-01	0.95	1.93E-01	7.14E-01	-0.25	7.16E-01	9.71E-01
16130	pgant8	1.04	2.40E-01	1.12	8.70E-02	6.42E-01	0.07	9.03E-01	9.94E-01
2562	CG30487	1.60	2.40E-01	1.14	2.37E-01	7.36E-01	-0.46	6.23E-01	9.56E-01
5473	kek5	-2.01	2.41E-01	-0.15	8.97E-01	9.81E-01	1.86	1.34E-01	7.54E-01
18897	Oaz	1.28	2.44E-01	0.66	3.86E-01	8.09E-01	-0.61	4.19E-01	9.12E-01
2284	mir-125	1.20	2.45E-01	0.87	2.36E-01	7.36E-01	-0.33	6.38E-01	9.56E-01

3572	CG13046	1.35	2.45E-01	0.60	4.58E-01	8.40E-01	-0.76	3.53E-01	8.93E-01
3508	stv	1.60	2.49E-01	1.61	1.11E-01	6.65E-01	0.02	9.87E-01	9.99E-01
22925	CG9083	1.07	2.54E-01	0.95	1.61E-01	7.02E-01	-0.12	8.46E-01	9.88E-01
2601	Ccp84Af	1.41	2.54E-01	0.55	5.25E-01	8.66E-01	-0.87	3.18E-01	8.82E-01
3365	fru	1.91	2.54E-01	1.96	1.08E-01	6.63E-01	0.06	9.60E-01	9.97E-01
3090	CG34117	1.33	2.56E-01	0.50	5.33E-01	8.70E-01	-0.82	3.14E-01	8.80E-01
8180	CG14658	-1.27	2.61E-01	-1.17	1.53E-01	6.97E-01	0.10	8.93E-01	9.93E-01
17744	CG31465	-1.00	2.61E-01	-0.08	8.91E-01	9.80E-01	0.92	1.53E-01	7.77E-01
2018	Lcp1	1.95	2.63E-01	1.90	1.32E-01	6.81E-01	-0.05	9.68E-01	9.97E-01
1391	VGAT	1.83	2.63E-01	1.00	3.80E-01	8.06E-01	-0.83	4.66E-01	9.25E-01
7681	CG31226	-1.32	2.63E-01	-0.02	9.77E-01	9.96E-01	1.30	1.30E-01	7.50E-01
29366	Ir67b	1.10	2.65E-01	0.99	1.65E-01	7.04E-01	-0.11	8.66E-01	9.91E-01
885	CG7255	-1.08	2.65E-01	0.00	9.97E-01	1.00E+00	1.08	1.26E-01	7.44E-01
1197	Npc1a	1.48	2.66E-01	1.21	2.03E-01	7.20E-01	-0.27	7.67E-01	9.78E-01
1834	dib	1.56	2.69E-01	1.06	2.84E-01	7.59E-01	-0.49	6.12E-01	9.53E-01
1646	CG6696	1.43	2.69E-01	1.27	1.71E-01	7.04E-01	-0.16	8.60E-01	9.91E-01
3371	CG15398	1.86	2.73E-01	1.89	1.25E-01	6.77E-01	0.04	9.75E-01	9.98E-01
8070	AttA	-1.14	2.75E-01	-7.34	7.69E-07	3.18E-04	-6.20	3.77E-06	9.18E-04
3509	CG2127	2.26	2.75E-01	2.34	1.21E-01	6.74E-01	0.08	9.54E-01	9.96E-01
6430	CG10035	-1.34	2.76E-01	0.04	9.65E-01	9.93E-01	1.38	1.24E-01	7.43E-01
3696	Nup50	-1.10	2.78E-01	-1.03	1.59E-01	7.02E-01	0.07	9.20E-01	9.95E-01
2428	Ddr	1.69	2.79E-01	1.20	2.76E-01	7.55E-01	-0.48	6.53E-01	9.60E-01
1807	Spn	1.91	2.85E-01	1.84	1.54E-01	6.98E-01	-0.07	9.55E-01	9.96E-01
1939	roX1	1.06	2.86E-01	0.91	2.03E-01	7.20E-01	-0.15	8.23E-01	9.86E-01
2565	CG17139	1.77	2.93E-01	1.43	2.34E-01	7.35E-01	-0.34	7.67E-01	9.78E-01
1716	CG34388	1.05	3.02E-01	0.79	2.72E-01	7.53E-01	-0.26	7.14E-01	9.71E-01
971	AttC	-1.08	3.02E-01	-6.13	4.46E-06	1.17E-03	-5.05	2.56E-05	3.79E-03
1683	CG12849	1.63	3.03E-01	0.70	5.28E-01	8.68E-01	-0.94	3.98E-01	9.06E-01
2598	CG42650	2.00	3.03E-01	1.82	1.92E-01	7.12E-01	-0.18	8.94E-01	9.93E-01
1648	CG11697	1.07	3.03E-01	1.08	1.50E-01	6.95E-01	0.02	9.82E-01	9.99E-01
652	CG1552	1.08	3.04E-01	-0.23	7.56E-01	9.44E-01	-1.30	9.30E-02	6.86E-01
1311	tth	-1.01	3.14E-01	-0.78	2.71E-01	7.52E-01	0.22	7.45E-01	9.74E-01
4300	AttA	-1.13	3.19E-01	-7.85	9.14E-07	3.42E-04	-6.72	3.93E-06	9.44E-04
1586	metl	-1.04	3.24E-01	-1.04	1.76E-01	7.06E-01	0.01	9.93E-01	9.99E-01
2513	CAP	1.05	3.30E-01	1.00	2.00E-01	7.18E-01	-0.06	9.40E-01	9.96E-01
2200	btd	1.33	3.30E-01	0.69	4.67E-01	8.42E-01	-0.63	5.05E-01	9.34E-01
1858	CG17304	1.20	3.31E-01	0.72	4.08E-01	8.19E-01	-0.48	5.74E-01	9.46E-01
13962	CG10375	1.52	3.31E-01	1.69	1.40E-01	6.86E-01	0.17	8.77E-01	9.93E-01
2688	CG14072	1.39	3.32E-01	1.09	2.85E-01	7.59E-01	-0.30	7.61E-01	9.77E-01
2581	α-Est2	1.31	3.32E-01	1.30	1.83E-01	7.08E-01	-0.01	9.91E-01	9.99E-01
10745	CG43755	1.52	3.35E-01	1.78	1.24E-01	6.77E-01	0.26	8.13E-01	9.85E-01
461	CG14671	1.17	3.42E-01	0.14	8.68E-01	9.74E-01	-1.03	2.43E-01	8.42E-01
2249	CG12866	1.17	3.46E-01	0.77	3.78E-01	8.05E-01	-0.40	6.44E-01	9.58E-01
626	CG8157	1.01	3.49E-01	-0.17	8.17E-01	9.61E-01	-1.19	1.35E-01	7.54E-01
15325	kl-5	1.06	3.51E-01	0.53	5.07E-01	8.58E-01	-0.53	5.03E-01	9.34E-01
575	ATPsyn-gamma	1.14	3.53E-01	-0.01	9.92E-01	9.98E-01	-1.15	1.95E-01	8.14E-01
27879	AttB	-1.08	3.55E-01	-7.49	1.90E-06	5.92E-04	-6.41	8.00E-06	1.61E-03
2260	CG17601	1.62	3.56E-01	1.06	3.92E-01	8.11E-01	-0.56	6.46E-01	9.58E-01
2521	CG13748	1.19	3.59E-01	0.97	2.91E-01	7.62E-01	-0.21	8.11E-01	9.84E-01
20957	Diedel	-1.26	3.64E-01	-4.91	3.45E-04	2.88E-02	-3.65	2.85E-03	1.15E-01
642	CG33308	1.01	3.65E-01	0.29	7.03E-01	9.31E-01	-0.71	3.65E-01	8.97E-01
641	CG7414	1.00	3.67E-01	-0.22	7.73E-01	9.50E-01	-1.22	1.33E-01	7.53E-01
2582	ImpL1	1.49	3.82E-01	1.37	2.62E-01	7.49E-01	-0.12	9.20E-01	9.95E-01
19060	lms	1.05	3.85E-01	0.72	3.98E-01	8.14E-01	-0.33	6.97E-01	9.68E-01
2539	skpF	1.29	3.86E-01	1.31	2.20E-01	7.30E-01	0.02	9.82E-01	9.99E-01
1388	CG1690	1.35	3.90E-01	1.27	2.59E-01	7.46E-01	-0.08	9.41E-01	9.96E-01
393	c(3)G	1.03	3.94E-01	-0.12	8.84E-01	9.78E-01	-1.15	1.89E-01	8.10E-01
1687	CG3544	1.49	3.98E-01	0.93	4.52E-01	8.37E-01	-0.55	6.52E-01	9.59E-01
1352	CG15198	-1.17	3.99E-01	-0.67	4.89E-01	8.51E-01	0.49	6.10E-01	9.53E-01
2033	CG5379	1.67	3.99E-01	1.05	4.53E-01	8.38E-01	-0.62	6.53E-01	9.60E-01
5172	CG41454	1.18	4.08E-01	1.19	2.48E-01	7.43E-01	0.01	9.96E-01	9.99E-01
1239	Jheh1	1.06	4.12E-01	-0.25	7.76E-01	9.51E-01	-1.31	1.63E-01	7.88E-01
1652	CG12535	1.06	4.13E-01	1.34	1.58E-01	7.02E-01	0.28	7.59E-01	9.77E-01
2423	betaNACtes6	1.02	4.31E-01	1.29	1.73E-01	7.04E-01	0.27	7.67E-01	9.78E-01
1291	Tsp33B	1.02	4.49E-01	1.13	2.46E-01	7.41E-01	0.11	9.09E-01	9.94E-01
1768	CG10899	1.15	4.85E-01	1.34	2.58E-01	7.46E-01	0.19	8.67E-01	9.91E-01
95	CG4970	1.14	4.87E-01	0.32	7.81E-01	9.51E-01	-0.82	4.79E-01	9.29E-01
1999	CG34181	1.21	4.91E-01	1.60	2.12E-01	7.25E-01	0.39	7.54E-01	9.76E-01
934	CG12376	-1.43	4.92E-01	0.42	7.71E-01	9.50E-01	1.85	2.20E-01	8.26E-01
2380	CG5389	1.14	5.11E-01	0.80	5.12E-01	8.61E-01	-0.34	7.82E-01	9.81E-01

22267	CG14013	1.05	5.12E-01	1.61	1.71E-01	7.04E-01	0.56	6.20E-01	9.55E-01
2365	CG14654	1.05	5.12E-01	0.99	3.85E-01	8.08E-01	-0.06	9.59E-01	9.97E-01
2136	CG12589	1.04	5.16E-01	1.37	2.39E-01	7.39E-01	0.33	7.72E-01	9.79E-01
1448	Sur	1.10	5.46E-01	1.58	2.32E-01	7.34E-01	0.48	7.06E-01	9.69E-01
2191	nAcRbeta-96A	1.25	5.55E-01	0.75	6.16E-01	9.02E-01	-0.50	7.37E-01	9.73E-01
2039	CG14372	1.02	5.80E-01	0.03	9.84E-01	9.97E-01	-1.00	4.49E-01	9.21E-01
2087	CG4393	1.32	5.87E-01	1.25	4.69E-01	8.43E-01	-0.07	9.68E-01	9.97E-01
1659	CG8160	1.12	6.02E-01	2.02	2.00E-01	7.18E-01	0.90	5.56E-01	9.44E-01

Table B10: Genes with a difference of 1.0 or more between virgin and mated eggless females in the log fold change in transcript abundance after infection (Comparison A minus Comparison B from Figure 5.1). This table contains the difference between Comparison A and Comparison B and also the corresponding Comparison A and Comparison B values for each probe.

ProbeUID	Gene name	logFC: (vun-vinf)-(mun-minf)	Uncorrected p-value	logFC (vun-vinf)	p-value (vun-vinf)	B.H. adj. p-value (vun-vinf)	log FC (mun-minf)	p-value (mun-minf)	B.H. adj. p-value (mun-minf)
19212	CG31775	1.45	9.30E-04	-3.19	5.17E-08	2.17E-05	-4.65	1.34E-09	1.88E-06
3102	CG31775	1.06	1.90E-03	-2.04	4.98E-07	1.09E-04	-3.10	9.00E-09	5.40E-06
363	CG30281	1.21	4.26E-03	-0.01	9.82E-01	9.96E-01	-1.21	3.86E-04	1.55E-02
19132	CG4830	-1.15	5.54E-03	0.16	5.12E-01	8.32E-01	1.31	2.09E-04	1.00E-02
23013	Try29F	1.41	7.51E-03	2.09	3.62E-05	2.46E-03	0.69	4.46E-02	3.44E-01
20602	Try29F	1.35	1.28E-02	2.07	6.52E-05	3.70E-03	0.72	4.72E-02	3.55E-01
20387	CG5778	-1.46	2.34E-02	-0.33	4.18E-01	7.77E-01	1.14	1.49E-02	1.78E-01
8032	CG11854	1.16	2.96E-02	2.25	4.01E-05	2.65E-03	1.09	7.42E-03	1.13E-01
27279	to	1.03	9.23E-02	2.37	1.18E-04	5.73E-03	1.35	6.26E-03	1.01E-01
1011	CG13527	-1.26	1.42E-01	-0.69	2.43E-01	6.39E-01	0.56	3.36E-01	8.25E-01
13016	His4:CG33891	-1.41	1.70E-01	0.07	9.25E-01	9.84E-01	1.48	5.34E-02	3.82E-01
615	Cct1	-1.16	1.82E-01	-0.20	7.30E-01	9.27E-01	0.96	1.25E-01	5.84E-01
972	CG17490	-1.38	1.91E-01	-1.03	1.69E-01	5.52E-01	0.35	6.28E-01	9.53E-01
2806	CG32850	-1.40	2.18E-01	-1.22	1.38E-01	5.08E-01	0.19	8.10E-01	9.85E-01
21321	ms(2)34Fe	-1.10	2.33E-01	-1.08	1.09E-01	4.59E-01	0.02	9.73E-01	9.99E-01
14162	l(1)1Bi	-1.32	2.36E-01	-0.14	8.55E-01	9.67E-01	1.18	1.42E-01	6.11E-01
1172	CG7140	-1.50	2.39E-01	-0.16	8.54E-01	9.67E-01	1.34	1.44E-01	6.15E-01
889	RpL21	1.18	2.50E-01	-0.11	8.79E-01	9.73E-01	-1.28	8.90E-02	4.99E-01
249	ETHR	1.26	2.50E-01	0.07	9.27E-01	9.84E-01	-1.19	1.34E-01	5.98E-01
2964	CG31414	-1.13	2.63E-01	-0.87	2.28E-01	6.20E-01	0.27	7.03E-01	9.70E-01
1121	CR43649	-1.15	2.71E-01	-0.06	9.38E-01	9.86E-01	1.10	1.48E-01	6.20E-01
26644	Eaf	1.03	2.90E-01	-0.14	8.37E-01	9.62E-01	-1.17	1.03E-01	5.36E-01
3115	CG11663	-1.48	2.91E-01	-1.49	1.46E-01	5.20E-01	0.00	9.99E-01	1.00E+00
14028	CG31698	-1.31	2.98E-01	0.00	1.00E+00	1.00E+00	1.31	1.51E-01	6.25E-01
3123	CG32971	-1.04	3.02E-01	-1.01	1.64E-01	5.45E-01	0.02	9.73E-01	9.99E-01
2653	CG5755	-1.37	3.05E-01	-1.38	1.54E-01	5.31E-01	-0.01	9.91E-01	9.99E-01
1173	CecB	-1.34	3.05E-01	-5.04	1.80E-04	7.69E-03	-3.70	1.72E-03	4.36E-02
26645	pirk	2.07	3.07E-01	-2.98	5.37E-02	3.21E-01	-5.05	4.05E-03	7.67E-02
14030	Drep-3	-1.72	3.08E-01	0.15	8.98E-01	9.78E-01	1.87	1.30E-01	5.92E-01
2962	bi	-1.43	3.09E-01	-1.37	1.78E-01	5.63E-01	0.06	9.48E-01	9.98E-01
3117	CG33475	-1.37	3.27E-01	-1.35	1.81E-01	5.66E-01	0.02	9.86E-01	9.99E-01
4915	CG34205	-1.09	3.34E-01	-0.10	8.93E-01	9.77E-01	0.98	2.24E-01	7.25E-01
1153	Ero1L	1.16	3.42E-01	-0.50	5.53E-01	8.53E-01	-1.67	7.05E-02	4.46E-01
253	CG32846	-1.16	3.80E-01	-0.49	5.91E-01	8.69E-01	0.66	4.74E-01	9.01E-01